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Abstracts for a conference on trace elements in diet, nutrition, and health: essentiality and toxicity

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Category 1: Trace element intakes, dietary patterns, bioavailability, and tissue distributions

1.P01

The effect of enrichment with sunflower seed, sesame seed and alpha tocopherol acetate to linoleic acid quantity in cookies

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The study investigates the nutrients and oxidative effect of enrichment with sunflower seed, sesame seed, and alpha tocopherol acetate to linoleic acid quantity in cookie. Chemical analysis for total fat (gas-chromatography: GC), cholesterol (GC), protein (Kjedahl method), iron (atomic absorption) were analyzed by Agro-Industry Development Center for Export, Prince of Songkla University (PSU), and linoleic acid (GC: esterification) was analyzed by the Food Science and Nutrition Department, Faculty of Science and Technology PSU, Thailand. Sunflower and sesame seeds were mixed with wheat flour in proportions of 10:10, 10:20, 10:30, 20:10, 20:20, 20:30, 30:10, 30:20, and 30:30%, respectively. Sensory acceptability in the studied cookies was measured. Alpha tocopherol acetate was added in cookie. The oxidative effect of enrichment with sunflower seed, sesame seed, and alpha tocopherol acetate to linoleic acid in cookie was evaluated by the change of the rate of oxidation in thiobarbituric acids (TBARS) and peroxide value. Results showed that cookies enriched with sunflower and sesame seed in the proportion of 20:10% with 1,500 ppm alpha tocopherol acetate were significantly better in smell, taste, texture, and preference ($p<0.05$). Nutrients in 100 g of cookies with 20% of sunflower seed and 10% of sesame seed consists of 545.53 calories, 8.92 g protein, 32.21 g total fat, 13.30 mg cholesterol, 56.49 g carbohydrate, 336 mg sodium, 1.6 mg iron, and 25.6 mg of linoleic acid. The expanding rate was 1.06+0.02 cm, and hardness was 257.06+1.49 g. Color L*, a*, and b* was 69.93+0.82, 5.31+0.59, and 22.98+0.48, respectively. The addition of 1,500 ppm alpha tocopherol acetate to cookies had more linoleic acid than the controls. Alpha tocopherol acetate can reduce oxidation reaction of linoleic acid in cookies. This kind of cookie consists of some amount of iron.

1.P02

Estimation of the potentially bioavailable fraction of copper and arsenic in soils by DGT

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The existing Italian and European regulation concerning agricultural soils is based on a risk assessment procedure that takes into account only the total concentration of heavy metals. This conflicts with the well-known assumption that the concentration to which organisms and plants are exposed is represented by the "bioavailable" fraction. The fraction bioavailable to plants is the amount that can be taken up by the roots of a given plant species and strongly depends on its oxidation state, on the chemical form, on soil properties, and on the root activity. The technique of diffusive gradients in thin films (DGT) is by now recognized as a tool to measure the potentially available concentration in soil solution and as a mime for plant uptake. This work represents a survey of two different investigations, realized separately to study the bioavailability of copper and arsenic in two different case studies, all represented by Italian soils, polluted by long-term human activities (agricultural and industrial, respectively). In all cases, even if concentration of metals in soils was very high and, according to the law, unacceptable for crop cultivation, the soluble and the bioavailable concentrations were very low, suggesting an unlikely accumulation in crops. Subsequently, for copper and arsenic, we also investigated the mobilization induced in the rhizosphere by a tolerant and a hyperaccumulator plant, respectively (*Zea mays* and *Pteris vittata*). All the experiments were performed by DGT.

1.P03

Research studies related to the impact of environmental exposure to boron on people living in Turkiye

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Turkiye has the world's largest borate deposits and is the leading boron ore producer in the world. National Boron Research Institute (BOREN) was established under the authority of Ministry of Energy and National Resources. BOREN aims to increase consumption of boron products in Turkiye and to develop and support R&D studies for new potential sources. Another scope of BOREN is to form a databank and documentation centre for the people interested in boron products and all related fields. For this purpose; questionnaires, national/ international workshops, symposiums and discussion meetings have been arranged. The Institute is taking a role as a bridge between universities, research organizations, and pub-

lic/private sector companies. Boron is a naturally occurring trace element and is ubiquitous in the human environment. Some investigators have reported that boron is essential for human health. Although the mechanism of boron in humans is not understood clearly, it is known that boron has an important role in calcium and magnesium metabolism, bone growth, and insulin metabolism. On the other hand, on the basis of some results from animal tests, it has been claimed that high intakes of boron have a toxic effect on reproductive function in humans. These conclusions are controversial, as the high boron levels used in animal studies are not equivalent to typical human dietary boron intakes. However, inhalation of boron-containing dust during the process of boron mining, subsequent industrial processing, or manufacture of boron-containing products may increase body boron levels. From this perspective, BOREN has supported new boron research projects related to animal boron nutrition, human male fertility, and the prevention of prostate cancer. Situated in the world's largest natural boron laboratory area, BOREN is equipped uniquely to conduct new boron health studies with interested collaborators.

1.P04

Molybdenum in the environment in connection with biological effects

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The level of molybdenum and copper concentrations in organisms (plants, lichens, mushrooms, hair of cattle) under conditions of opened deposits and background territories of East Transbaikalia and Northern Caucasus was investigated. Copper and molybdenum were determined by means of AAS and ICP-MS. The method precision was controlled through standard soil samples (USA, Poland, China). It is established that on a level of molybdenum and its accumulation by plants, landscapes of Tyrni-Auz Mo-W-mining exceed the natural–man-made biogeochenoses of East Transbaikalia (Bugdaya, Verkhniya Shakhtama) appreciably. Indicator value have the cow vetch—*Vicia crassa*, the larch—*Larix sibirica* (needles), some groups of mosses and lichens, and to a lesser degree—leaves of the birch—*Betula alba*, mushrooms and seaweed on accumulation of Mo and Cu. At an intoxication of cattle by copper compounds, concentrations of molybdenum in hair are sharply reduced, and diagnostics of Mo- and Cu-microelementhoses can be carried out under the contents of these metals in hair cover (supported by grant of RFFR 05-05-64547).

1.P05

Assessments of exposure to dietary trace elements in China

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To assess the safety and nutritional status of dietary trace elements in different areas in China, the dietary intakes of 15 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 in 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/day (36.1%), 0.022 mg/day (35.3%), 0.007 mg/day (15.2%), 22.9 mg/day (36.3%), 0.276 mg/day, and 0.079 mg/day (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 (percent of AI or RNI) in adult males were 13.0 mg/day (87.0%), 10.4 mg/dY (69.3%), 1.7 mg/day (84.5%), 0.064 mg/day (128.6%), 3.8 mg/day (127.9%), 0.138 mg/day (276.4%), 0.257 mg/day (428.9%), 0.242 mg/day, 0.034 mg/day, and 0.053 mg/day, respectively. The dietary intakes of selenium were higher than RNI in most provinces, but the intakes in Ningxia (0.037 mg/day) and Jiangxi (0.040 mg/day) 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, and 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.

1.P06
Open

1.P07
Beneficial role of monothiols along with magnesium in the mobilization of mercury: a possible mechanism

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Chronic exposure of mercurial organic compounds is a worldwide health concern and could be pathogenetically relevant as cofactor in several neurodegenerative diseases. Occupational exposure to methyl mercury in humans is 0.026 mg/Hg/day and causes neurological symptoms. The aim of the present study is to develop a companion formula of chelator along with antioxidants as an intervention strategy and thus a practical solution to mercury intoxication. The present investigation relates to the exposure of methylmercury in rats. The efficacy of exogenous applications of glutathione (0.3 mM/kg, i.p.), D-penicillamine (50 mg/kg, p.o.) in combination with magnesium (10 mg/kg, p.o.) in the acceleration of sub-chronic mercury (1.5 mg/kg, p.o.) elimination and in the reversal of intoxication has been discussed. Administration of mercury (5 days/week for 21 days) produces significant rise in the serum enzymatic activities (AST, ALT, SALP, and LDH; $p<0.05$). A rise was found in hepatic, renal, and neuronal lipid peroxidation with a concomitant decline in reduced glutathione ($p<0.05$). The enzymatic activities of GSH cycle, such as GPx, GR, and G-6PDH were also altered due to oxidative damage. A fall was observed in the enzymatic activity of AChE in fore-, mid-, and hindbrain. Results revealed that the combination of GSH + Mg (5 days posttreatment) proved to be the most efficacious in mobilizing mercury and helped in the recovery of biochemical variables. Light microscopic and ultrastructural changes also supported the above findings.

1.P08
Trace elements in the digestive system of the rat

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Trace elements play a significant role in the human body. Some of them are bounded covalent to proteins such as the selenoproteins, but other binding forms are also possible. Trace element-containing proteins are involved in metabolic processes. Therefore, their availability is indispensable in living form of life. In the present study, investigation with regard to the distribution of some trace elements in the gastrointestinal tract of the rat was carried out. As analytical instrument for this study, the instrumental neutron activation analysis (NAA) was used. After a suitable preparation of the samples for their irradiation by means of the neutrons which were obtained from the BER II, the concentrations of some trace elements were determined. The obtained results regarding the determination of the concentration of several trace elements in the digestive system indicate at first the importance of the elements in this tract and, on the other hand, provide information about the existence of trace element-containing proteins.

1.P09
Lysosomes of the gastric mucosa cells: a site of accumulation of aluminium and indium

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Aluminium (Al) and indium (In) are two neurotoxics used in both medicine and industry. Aluminium is present in drinking water, in antacid medicines and in antiperspirants. Indium is used as a dental alloy and as a radiotracer and in nuclear reactor, lamps and in jewelry. Aluminium and indium have been involved as causative factors in several clinical and neuropathological diseases like encephalopathy, osteomalacia, asthma and pulmonary fibrosis after parenteral administration. In the present work, we have attempted to study the behaviour of these elements in the gastric mucosa and the liver after their intragastric administration, using the conventional transmission electron

microscopy. The ultrastructural study showed that aluminium and indium were concentrated as electron dense granulations in the lysosomes of the principal and parietal cells of the gastric mucosa. No aluminium or indium accumulation was detected in the various studied hepatic territories. Aluminium and indium deposits were never observed in stomach and liver cells of the control rats. The mechanism of concentration and insolubilisation of mineral elements in the lysosomes can be considered as a physiological process to protect the organism against invasion of toxics in the blood.

1.P10

Mixed exposure to nephrotoxic metals in a Bangladeshi population with prevalent malnourishment and risks for kidney effects

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Arsenic, cadmium, inorganic mercury, lead and uranium are known to be nephrotoxic at high exposure levels. Although people often are exposed to several of the metals, the potential health effects have almost exclusively been evaluated for the metals one by one. Very few studies have addressed the possible interactive effects of combined exposure or the modifying effect of malnutrition. Recently, early signs of renal dysfunction were observed for both adults and children after simultaneous environmental exposure to rather low exposure levels of cadmium and arsenic. Therefore, we decided to investigate the possible interactive effects on kidney function of long-term, low-level multimetal exposure in Matlab, Bangladesh, where we previously have found elevated concentrations of arsenic in drinking water and that people are potentially exposed to cadmium and lead via foods (rice is main staple food) and inhalation at various levels depending on water source, food habits and activity patterns. Urine and serum samples that were previously collected within the AsMat study (Arsenic in tube-well water and health consequences in Matlab) in Bangladesh were analysed for biomarkers of metal exposure, early renal effects (U-RBP, U-Alb; S-Cystatin) and nutrition (S-fer, Zn, Se, vit A, BMI). Information on potential covariates such as age, gender, socioeconomic status, diabetes, etc. was included in the analysis. The study group comprises about 1,000 individuals; 700 men and women above 30 years of age, 100 adolescents 14–15 years of age and 200 children 8–12 years of age, randomly selected from the Matlab population. Preliminary results indicate elevated exposure to arsenic, cadmium and lead (median concentrations in urine 77, 0.60 and 2.5 μg/L, respectively) and prevalent malnutrition (37% of adults had a BMI<18.5 kg/m2). Financial support provided by WHO, SIDA, USAID, and PHIME is gratefully acknowledged.

1.P11

Contamination of selected food by heavy metals

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In our project, we evaluated content of cadmium, mercury and lead in selected groups of food. We analysed 1,946 samples of food. Samples were taken from several food companies covering the whole area of Slovakia during 2004, 2005 and 2006. Samples, 254, were analysed for cadmium content. In 2004, there were 100 samples (39.3%) below detection limit, mostly samples of wine (100%). Samples below detection limit were not from noodles, bread or rolls (0% below detection limit). The highest acceptable level was exceeded in 2 (mill products and dried fruits) of 12 kinds of food. In 2004, the highest acceptable level was exceeded only in 1 kind of food (sweets); 1 sample of 15 exceeded highest acceptable level (6.6%). Also, in 2006, the highest acceptable level was exceeded only in 1 kind of food (dried fruits) in 1 sample of 16 (6.2%). Samples, 633, were analyzed for mercury content. Samples of 12 kinds of food did not contain mercury content at about the highest acceptable level. During 2004, 2005 and 2006, 663 samples were analysed for lead content in 12 kinds of food. In this period, the highest acceptable level of lead content was exceeded in five groups of

food. In 2004, only one kind (fruits beverages), in 2005, two kinds (sweets and fruit beverages) and in 2006, two kinds (dried fruits and fruits beverages) had exceeded the highest acceptable level.

1.P12

Investigation of interrelationships between hair and whole blood macro and trace element contents in humans

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Multielement (Ca, Mg, P, K, Na, Fe, Zn, Cu, Mn, Co, Cr, Si, Se, Ni, Al, As, Cd, Hg, Pb) analysis of scalp hair and whole blood of 173 healthy subjects (97 males, 76 females; 1–49 years old) were performed by combined ICP-OES/ICP-MS. The samples were treated by m.w. digestion; CRM GBW09101 and ClinCheck 8840-8843 were used for quality control. According to Pearson (*P*) and Spearman (*S*) correlation, hair Pb finely corresponds with whole blood Pb concentration (r=0.59–0.78 *P*, 0.35–0.82 *S* in males and 0.50–0.63 *P*, 0.81 *S* in females). In different age and sex groups, the most common were positive correlations for Cd (r=0.49–0.89), Ni (0.35–0.68), Co (0.33–0.54). In 4–6 years old boys. there were significant correlations for Zn (r=0.41 *S*, 0.43 *P*), Ni (0.41 *S*, 0.68 *P*), Pb (0.72 *P*), Co (0.60 *P*), and As (−0.40 *S*, *P*). In 1–3 year old boys, negative correlations for *P* (r=−0.78 *S*, −0.77 *P*) were found. In 1–6 year old girls, such group of elements included besides Pb, Ni, Co, Cd, also Fe (r=0.61 *P*), Mn (0.50 *P*), and Se (0.55 *P*). In 18–34 year old males, significant positive correlations between hair and blood Hg, Pb, Cu (r=0.35 S) and the negative one in case of Cr (r=-0.35 S) were found. In 18–34 year old females, there were close interrelationships for Ca (r=−0.90 S), Cd (0.89 S). In 35–49 year old males, hair Pb (r=0.82 *S*) and Cd (0.89 *S*) significantly correlated with their blood concentrations. In females, there were strong correlations for Cd (r=0.63 *S*, 1.00 *P*), Hg (0.96 *P*), Pb (0.81 *S*), P (0.94 *P*), Zn (0.89 *P*). General considerations are: (a) hair can reflect the body content of Pb, Cd, Hg, As, Co, Ni, Zn, Al, P, Cr, Cu, Se, Fe, Ca; (b) hair and whole blood elemental content correlations depend on age and sex; (c) the correlations are more prominent in children; (d) hair and blood element contents of main toxic metals (Pb, Cd) correspond closely in any age and sex. So, human hair multielement analysis can be recommended widely as a diagnostic tool for noninvasive assessment of body macro- and trace element status.

1.P13

A nationwide survey of trace elements in lynx, wolverines, wolves, and brown bears in Norway

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Since 1993 in Norway, the Directorate for Nature Management has systematically registered all individuals of the large predator brown bear (*Ursus arctos*), grey wolf (*Canis lupus*), lynx (*Lynx lynx*), and wolverine (*Gulo gulo*) that have been either shot, hit by a car, or found dead. By 2006, the resulting database encompassed 1,464 animals. Considerable efforts are continuously made to identify as many animals as possible from these four species and to collect tissue samples when practically possible. From the 1,464 animals, we carefully selected approximately 150 individuals of lynx and 100 wolverines, on the basis of age, sex, and geographical distribution. All individuals of bears (29) and wolves (39) where tissues existed were included in the study. We are not aware of any published similar large-scale systematic studies of trace elements in large predators worldwide. A few studies have been published on the contents of a limited number of metals in terrestrial mammals in Norway, mostly on herbivores such as moose and hare, but these have covered only a few subjects from restricted areas. Great care was taken to avoid contamination of the samples during preparation and analysis. Tissue samples of 0.7–1.3 g were

dissected from partially thawed liver, kidney, and muscle, using titanium knives on polyethene boards. The samples were then freeze–dried to constant weight to express the concentrations on a dry weight basis. The freeze–dried samples were decomposed with concentrated nitric acid in an UltraClave (microwave-based decomposition unit) before determination of more than 50 elements employing high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). Results will be presented at the conference.

1.P14

Iron–zinc–copper interactions in edible insect jumil bug *Euchistus taxcoensis* A

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Sixteen mineral elements are generally considered nutritionally essential for humans or as toxic in excessive concentrations; seven major or macrominerals (Ca, P, K, Na, Cl, Mg, and S) and another eight trace or microminerals (Fe, Zn, Cu, I, Mn, Se, Co, and Mo) should be included in a population regular diet. Mineral requirements and intake are affected by many factors; people age, chemical form of elements, interrelationship among them and with other nutrients; therefore, in the selection of food, the biological availability of the desired elements must be considered to obtain the suitable source for them. The aim of this study is to establish interaction factors that affect bioavailability and absorption of iron–zinc–copper minerals in humans. Iron deficiency is a common nutritional problem worldwide, ranging from effects on energy metabolism and immune function, to cognitive and motor development. Manifestations of zinc deficiency are growth retardation, dermatitis, infections, predisposition, and skeletal abnormalities. Copper deficiency causes hypochromic anemia and increased risk of infection. Element interactions occur in biological systems, when elements share the same absorptive pathways or when deficiency of one element affects the metabolism of another. Evaluation of Fe, Zn, Cu content in jumil bug *Euchistus taxcoensis* A was gathered November 2006, and analysis of the ash samples was performed by atomic absorption spectrophotometry. Data obtained were: iron 9.47 mg/100 g; zinc 8.97 mg/100 g; copper 0.59 mg/100 g. Mineral intake requirements for adults are Fe 10 mg/day; Zn 10 to 15 mg/day, Cu 1.5 mg/day. Elements mentioned interact with each other, but in jumil bugs, metal ratios are balanced. Interactions occur when the elements were given as a supplement with pharmacological preparations, and food fortifications or dosages of micronutrients are not considered; so excessive concentrations become toxic. Mineral content in jumil bugs may vary according to environmental conditions.

1.P15

Mercury in urine after dental restoration

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A major source for human exposure to inorganic mercury is dental amalgam. It has been established that amalgam fillings will slowly release mercury into the oral cavity. For a period of 1 year, one of us (SL) removed 27 amalgam surfaces during 11 sessions in the dentistry. The surfaces were replaced with composite material. Urine samples were collected 2 h before the dentist appointment, and a new sample was collected the next morning. The first 100 ml of urine was discarded and the following 100–150 ml was collected for analyses of creatinin and trace elements. Trace elements were measured using high resolution ICP-MS (Element 2). A decline in urinary mercury level was observed.

1.P16

Trace elements in blood from dental health personnel

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During 2006, a questionnaire survey of more than 2,000 employees in dental surgeries in the central part of Norway was performed. The main purpose of the study was to search for possible correlation between mercury exposure from the use of amalgam and a

range of health effects. A group of 52 persons were selected for a detailed interview. The selection criteria were based on self-reported assessment of work conditions and possible exposure. An exposure score was generated on the basis of these criteria, and the group was divided into low, medium, and high scores for possible exposure. Extensive use of copper-based amalgam was part of the exposure score. Most of the exposures to mercury amalgams were terminated several years ago, and the use of amalgam in dentistry is very sparse today. At the time of interview, a blood sample was taken and analyzed for 31 trace elements including mercury, selenium, and zinc using HR-ICP-MS (Element 2, Bremen, Germany). Results show that the ranges of blood concentrations in the three groups were quite large. There was no readily observable correlation between exposure score and blood concentration of mercury.

1.P17

Influence of organic selenium addition in feed ration on muscle tissue structure and meat quality in calves

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The effects of dietary intake of selenium on calf muscle tissue structure, carcass composition, feeding indicators, and quality of meat were investigated. In this work, Holstein-Friesian calf carcasses were evaluated. Animals were divided into two groups. The control group (1) was given feed ration without selenium, experimental group (2) received 600 ppm of selenium in feed ration daily during 60 days. Animals were slaughtered on experimental abattoir of RIAS Nitra after 24-h starvation. Samples from musculus longissimus thoracis (MLT) and musculus semimembranosus (MSM) for histological evaluation were taken within 30 min after slaughter. Muscle samples were immediately frozen in liquid nitrogen and histochemically processed. Indicators of carcass composition, meat quality, and feeding indicators were observed. Histological analysis show higher diameter of alpha white muscle fibers in MSM and MLD in control group, while alpha-red and beta-red muscle fibers were thicker in experimental group. A higher proportion of alpha-white muscle fibers in the control group (1) was obtained, while alpha-red and beta-red muscle fibers had a higher proportion in the experimental group (2). Differences between relative volumes of connective tissue and adipose tissue were nonsignificant. Similar results were found in MLT. These results demonstrate that organic selenium addition in feed ration have no significant effect on the histological structure of muscle in calves. Indicators of carcass composition and feeding indicators were not influenced by selenium supplementation. From technological indicators of meat quality, selenium supplementation significantly affected only water holding capacity.

1.P18

Environmental risks of potentially toxic metals mobilised from acid sulphate soils in Finland: a literature review

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Acid sulphate (AS) soils currently cover a total of approximately 17–24 million ha in the coastal regions worldwide, with major occurrences in Africa, eastern Australia, Asia and Latin America. In Europe, the largest AS soil occurrences are found in Finland (1,000–3,000 km^2) and Sweden (500–1,400 km^2). These soils develop where sulphide-rich sediments are exposed to atmospheric oxygen resulting in sulphide oxidation and thus formation of sulphuric acid within the soil. The oxidation and weathering of these sulphide-rich sediments, which have risen above the sea level as a result of isostatic land uplift, have occurred as a result of artificial farmland drainage. This gives rise to very acidic conditions (pH 2.5–4.5) and mobilization of potentially toxic metals (e.g., Co, Mn, Al) from soil minerals. It has recently been shown that much larger quantities of a number of potentially toxic metals are leached from these soils than from the entire Finnish industry taken together. Based on the alarmingly few studies that have been carried out, all indicate an extensive and serious effect on biota, including metal imbalances in crops and

elevated concentrations of Al and Zn in the milk of cows. Acid sulphate soils have significant, but spatially and temporally variable impacts on biodiversity and community structure of fish, benthic invertebrates and macrophytes, with numerous cases of mass fish kills along the Finnish coast. Furthermore, chronic exposure to high concentrations of metals pose an actual threat on human health. The potential risks for Alzheimer's and Parkinson's disease in AS soil landscapes are discussed.

1.P19

Levels of selected trace elements in blood of Czech school children and women

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One part of the Human Biomonitoring in the Czech Republic is focused on the determination of selected trace elements in blood of more vulnerable populations like school children or women. Blood samples, 1,454, of children aged 8–10 years and 846 blood samples in women were analyzed in the first monitored period 1996–2003. The second period since 2005 included 172 women, mean age of 34 years. In 2006, we analyzed blood from 399 children, mean age of 9.2 years. Informed consent was obtained from each adult and child's parents. Pb, Cd, Cu, and Zn were determined by ETAAS, Se by HGAAS, Hg by CVAAS. Data were processed using Unistat 5.1 and Statistica 7. In children, blood Pb levels (medians) in the particular years vary between 36 and 30 μg/l with no significant decreasing trend and slightly lower levels in girls. The current Czech reference value for blood Pb in children is 55 μg/l. In adults, significantly lower levels were observed in women (25 μg/l) than in men (35 μg/l); time-related descending trend and age-related increase was observed in both genders. The levels of Cd in blood of children were in more than 60% of samples below the detection limit, 0.3 μg/l. In non-smoking women, the levels (medians) in the particular years vary between 0.75 and 0.5 μg/l. The levels of Hg in blood (medians) of children in consecutive monitored years were 0.57, 0.39, 0.38, and 0.42 μg/l, respectively. In adults, the values increased with age; in women, they were significantly higher (Me 1.16 μg/l) than in men (Me 0.91 μg/l). The levels of Se in blood vary between 64 and 72 μg/l irrespective of gender. In adults, significant ascending trend was observed. The levels of Cu in blood were higher in females than in males. Increased values were observed in women taking oral contraceptives. The obtained levels of trace elements did not exceed the existing biological significant limit values. The levels of the toxic elements tend to gradually decline in time [partially funded by the FOOD-CT-2006-016253 (PHIME)].

1.P20

Trace elements in nutritional supplements

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Trace elements in 54 nutritional supplements were analysed by high-resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS, Element 2, Bremen, Germany). These products included mineral and trace element supplements, both as single and multielement preparations. The main objective was to evaluate whether the product composition was accurately represented by the product declaration. The products were also analyzed for undeclared essential or toxic elements. Product user directions were examined and compared to public dietary intake recommendations of the elements. The results from this study show that most supplements contained all the declared minerals and trace elements. However, there were several discrepancies between product declaration and measured values, including deviation from declared values and presence of undeclared elements. The observed deviations are unlikely to pose any risk of serious health problems for the consumer. User declarations for the majority of products were in concordance with public dietary recommendations. However, content deviations may in some cases lead to intake in moderate excess of recommended doses.

1.P21

Mineral and phytate contents of some complementary foods from Cameroon

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Inappropriate complementary feeding linked with scarcity of data on food composition is the main cause of malnutrition and micronutrients deficiencies among Cameroon children. This study assessed the content of eight minerals (Ca, Fe, Mg, Zn, Pb, Cd, Cr, and Ni) and phytates of 30 maize and Irish potato-based complementary foods. The analysis of minerals was done by F-AAS for Ca, Mg, Fe, and Zn and by GF-AAS for Cd, Pb, Cr, and Ni. Phytate content was determined by HPLC, and phytate/zinc molar ratio was calculated. From the analyses, mineral contents expressed in mg/100 g dry matter (DM) ranged, respectively, from 13.88 to 149.3, 3.56 to 9.39, 6.07 to 142.7, 0.20 to 2.58, for Ca, Fe, Mg, and Zn, respectively. Expressed in μg/100 g DM 2.67 to 120.07 (28.67±26.1 07); 0.052 to 1.87 (0.46±0.49); 0.67 to 98.85 (16.86±20.23); 1.78 to 804.52, Pb, Cd, Cr and Ni, respectively, and for phytates, ranged from 0.000 to 6.043, and phytate/zinc ratios ranged from 0.000 to 51.62. It appears that germination and fermentation reduced the level of phytates; however, the level of mineral content in the samples did not change significantly.

1.P22

Assessment of mineral content and acid extraction in maize-based complementary foods from Cameroon using HR-ICP-MS

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Complementary foods in developing countries like Cameroon are based mostly on cereals or tubers, characterized by bulk and poor nutrients density. Various treatments were used to improve nutritional value of 28 complementary traditional gruels. Content of 16 minerals (Fe, Zn, Ca, Mg, Mo, Mn, Cu, Cr, Al, Ni, Se, Sn, Cd, Pb, Hg, and As) and their acid extraction percentages were determined using HR-ICP-MS. Median content in μg/g were 62, 12, 433, 0.2, 239, 105, 50, 1, 1, and 1 for Fe, Zn, Mg, Mo, Mn, Al, Ca, Cu, Ni, and Pb, respectively. Expressed in ng/g, they were 40, 173, 30.2, 55, 9, and 98 for Sn, Cr, As, Se, Cd, and Hg. Median extraction percentage were 16, 14, 14, 14, 12, 11, 9.1, 7.3, 7.2, 3.8, 2.8, 2.3, 1.9, 1.7, 1, and 1% for Mg, Mn, Zn, Ca, Ni, Pb, Cu, As, Cd, Al, Se, Fe, Mo, Hg, Cr, and Sn, respectively. There were correlations between magnesium and zinc and between cadmium and molybdenum with the correlation coefficients over 0.9. Dehusking maize led to lower content of all the analyzed minerals except copper and aluminium. Germination leads to a reduction from 50 to 100% of the elements tin, lead, chromium, mercury, and iron. Manganese, calcium, nickel, and copper were reduced by less than 25%. There was no obvious difference in the content of zinc and magnesium. However, mineral extraction in germinated soya bean was very good.

1.P23

Effect of trace elements in a complex preparate (immunovet-HBMTM) on the absorption from the animal digestive tract

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Farm animals acquire the macro- and microelements via feedstuffs. Industrially compounded feeds are supplemented with macro- and microelement premixes according to the species, breeds, age, and production branch of animals. In premixes, the elements are presented overwhelmingly in the form of inorganic salts, and their rate of utilization is often unsatisfactory owing to miscellaneous reasons. A number of reports published recently gave account on the beneficial effects of metallo-chelates or proteinates as source of important elements in animal diets. Immunovet-HBMTM is a fermented wheat-germ extract with high content of minerals, organically bounded trace elements (Fe, Mn, Cu, Zn, Se), vitamins (A, E, D etc.) with prebiotic, immunostimu-

lant and growth promoting effects. The authors summarize their experimental results obtained with this complex preparate on large-scale poultry and swine operations. In average housing and management conditions, the Immunovet-HBMTM in a daily dose of 1 kg/t feedstuff can efficiently improve several production parameters (e.g., ADWG, FCR, etc.) of broiler and fattening pig production when offered from day 1 until the end of rearing or from weaning until slaughtering. Several components of the preparate, among others, the additional supplementation with microelements, may play a decisive role viz via enhancement of self-produced digestive enzymes and absorption of nutrients and biologically active materials. This natural growth promoter puts a smaller load onto the animal organism and environment and increases the value and safety of foods of animal origin.

1.P24

Effect of 1 year of iron/zinc fortification on body iron stores of children aged 1–4 years

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Iron deficiency, in spite of supplementation programs, continues to be highly prevalent in children. Food-based approaches for combating both iron and zinc deficiency are only feasible interventions. We evaluated milk as a vehicle for delivery of micronutrients and its impact on body iron stores among 1–4 years children. In a community-based, double-masked, randomized, controlled trial in a peri-urban north India, a total of 633 children were enrolled and randomly allocated to consume three feeds per day of either, fortified milk (FM, n=316) or same milk without fortification (WF, n=317). Fortified milk delivered 9.6 mg iron, 9.6 mg zinc (additional 7.8 mg zinc), 6.6 μg selenium (additional 4.2 μg), 0.3 mg copper (additional 0.27 mg), 1,110 IU vitamin A (additional 540 IU), 48 mg vitamin C (additional 40.2 mg), 8.1 mg vitamin E (additional 7.5 mg) per day. Milk preparations were provided by Fonterra Brands Ltd, New Zealand, and the intervention continued for 1 year. The venous blood sample was drawn at baseline and end-study; serum ferritin/sTfR levels were analyzed using ELISA, and the ratio of the serum transferrin receptor to serum ferritin was used to estimate body iron stores. Effects of intervention on body iron stores were assessed by estimating paired mean change between baseline and end study (FM=94; WF=102). At baseline, the mean body iron stores (mg/kg) were comparable across the study groups (FM, n=166: −4.2±3.5; WF, n=167: −3.5±3.8; p=0.09). The mean body iron stores significantly improved among children consuming fortified milk [Iron stores (mg/kg), difference of mean: 3.89 (95% CI: 2.74, 5.03), p<0.001]. Fortification of milk with iron and zinc resulted in significant improvement in body iron stores among preschool children. This study is registered as an International Standard Randomized Controlled Trial, number NCT00255385.

1.P25

Elevated levels of selenium in the typical diet of the Amazonian riparian population

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Fish is a nutritious food, but it accumulates many toxic substances, including mercury. As part of our efforts in the Brazilian Amazon to maximize nutritional input from fish and minimize toxic risk, we have been studying the role of selenium (Se), which may influence Hg toxicity. Se is a trace essential nutrient, yet deficit and excess in Se status are associated with health disorders. Se is ingested through food, notably fish, mammals, eggs, and certain nuts, whose content depends on soil Se concentrations and plant accumulation. Recent studies with riverside populations of the Tapajós River show highly variable and relatively elevated Se status, which varies among communities. The present study

was conducted in four communities of the Tapajós to evaluate Se sources in their typical diet and to examine relations between food Se concentrations and geographic location, dietary habits, local food production, and Se biomarkers. More than 650 food sources were sampled, and 201 persons participated in the study. Plasma Se, blood Se, hair Se, and urine Se were assessed. Food frequencies, agricultural production, and socio-demographic information were collected using an interview-administered questionnaire. Se analyses were performed by ICP-MS. Some foods such as eggs, meat, chicken, meat, and game meat contained considerable levels of Se (0.2–1.4 μg/g), while coconut, cacao, kale, sweet potatoes, and yucca had medium levels (0.2–0.1 μg/g). Brazil nuts (*Bertholletia excelsa*) contained very high levels of Se, from 12.1 to 62.6 μg/g. Geographical variations of food Se concentrations produced in different communities were observed. Blood Se levels presented a very large range (103–1,500 μg/L) and participants consuming Brazil nuts on a weekly or daily basis had the highest Se levels (558–1,500 μg/L). In this population with relatively high exposure to mercury, Se intake form local food may play an important role. Further studies will examine toxic effects of Se on human health.

1.P26

Study of bioavailability and toxicity of arsenic and mercury in Chinese propriety medicines (CPM) using an animal model

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Even though the Chinese propriety medicines (CPM) are widely used in many countries, there is a concern that they may cause adverse health effects from metal contaminants such as mercury (Hg) and arsenic (As). There has been little information on the study of metal bioavailability from CPM. The aim of this study was to investigate the bioavailability and toxic effects of As and Hg from CPM after short-term exposure using an animal model. Female Sprague–Dawley rats (24 rats, approximately 200 g, 6–7 weeks old) were used for this study. The rats were divided into six groups with four rats in each group. The four control (reference) groups were given sodium arsenate ($NaAsO_2$), arsenic sulphide (As_2S_3), mercuric chloride ($HgCl_2$) and mercuric sulphide (HgS), respectively. The other two groups were given CPM which were found to contain high As and Hg (Liu Shen Wan: arsenic 8.8% and mercury 1.5%; Niuhang Jie du Pian: As 6.5% and Hg <1.0 mg/kg). At the beginning of the experiment, all rats were given a single dose by oral gavage containing As or Hg at 5 mg/kg/body weight. Samples of urine and faeces were collected every 24 h for 9 days. After 9 days, the rats were killed and samples of liver and kidney were collected for analysis and histological assay. The results indicated that relatively high levels of arsenic from CPM were retained in liver and kidney, but only low levels of mercury were found in these tissues. The levels of arsenic from CPM were consistent with the levels from the arsenic-sulphide-treated group. With the exception of the mercuric chloride group, the levels of Hg in urine from other groups were very low. The elimination of As and Hg from the rat body tissues after CPM treatment will further be confirmed with the analysis of As and Hg in rat faecal samples. Histopathological examination of kidney and liver tissues did not show toxic effects from CPM.

1.P27

Iron bioavailability from grasshoppers: edible insects vs spinach leaves

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Iron-deficiency anemia is still a major nutritional problem in vulnerable population groups in the world because it can lead to negative changes in psychomotor and mental development, which may be irreversible. Animal food provides heme iron ready to be absorb; non-heme iron from plant sources is much less absorbable. Iron absorption is influenced by the dietary content, bioavailability of dietary, and amount of iron stored in the body. Non-heme and

heme iron are absorbed from the diet by different mechanisms. Non-heme iron from spinach plant are in the inorganic form as salts not solubilized, inhibited by phytates, polyphenols, and tannins among others and can be immobilized when bound up with other molecules. Heme iron from grasshoppers (edible insect) is in organic form, bind up with amino acids or proteins, and are without electronic charge. This study was conducted to determine properties of heme and non-heme iron from grasshoppers (edible insects) and spinach plant both obtained from the same environment in January 2007. Iron content in ash samples of grasshoppers, *Sphenarium purpurascens* Ch., and spinach leaves were performed by atomic absorption spectrophotometry. Results were for grasshoppers, 5.50 mg/100 g, and spinach leaves, 4.40 mg/100 g. In heme iron from animal sources, the molecule is bound within the porphyrin ring structure, is soluble in an alkaline environment; so, no binding proteins are needed for its luminal absorption. Non-heme iron in the lumen of the gut has variable solubility depending on the iron-binding compounds. There are two fundamental regulators of the amount of iron absorbed by humans. The total amount and form of iron compounds ingested, reflects the physio-chemical properties of the diet and the iron status of the individual. Spinach leaves have less amount of iron than grasshoppers, and in a regular diet, non-heme iron is absorbed at 5 to 10% efficiency and heme iron at nearly 40%.

1.P28

The element composition of cow's milk of Moscow region

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The element composition (25 elements) of milk of Black Motley cows from the Moscow region has been studied by ICP-AES and ICP-S methods. The average levels of essential and toxic chemical elements in cow's milk are found to be as follows (mg\kg): K (1,161)>Ca (1,041)>Na (1,025)>P (708)>Mg (109)> Zn (2.95)>Si (0.56)>Sr (0.51)>Fe (0.35)>B (0.17)> Al (0,09)>J (0.074)>Cr (0.048)>Cu (0.045)>Ni (0.045)>Se (0.039)>Mn (0.015)>V (0.013)>Li (0.012)>As (0.006)>Sn (0.003)>Co (0.002)>Pb (0.0015)>Hg (0.0005)>Cd (0.002). It is shown that the element composition of milk changes depending on type of feeding and biogeochemical features of facilities territory.

1.P29

Arsenic excretion in healthy, nonsmoking adult Norwegians: a randomised controlled seafood diet trial

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Norwegian adults are exposed to arsenic (As) mainly through seafood, mostly present in the nontoxic, organic arsenobetaine form. To assess the toxicological impact of As intake from seafood on human health, specified data on exposure and excretion of As species are required. The purpose of this study was to investigate whether the ratio between total As (tAs) and inorganic As (iAs) species in the urine after consuming a single As-rich meal with selected seafood reflects the ratio in the consumed diet and to examine how rapidly tAs is cleared from urine and blood plasma. Thirty-eight (28 women, 10 men) healthy, nonsmoking Norwegians aged 20–40 years were included in the controlled diet trial and randomised into four groups. After a 7-day run-in period where the subjects refrained from food items high in As (seafood, mushrooms, rice/rice products, and dietary supplements), each group consumed an As-rich meal containing either 150 g of cod (754 μg tAs, <5 μg iAs), blue mussel (632 μg tAs, 14 μg iAs), salmon (167 μg tAs, <5 μg iAs) or potato (control; <5 μg tAs, <5 μg iAs). All urine was collected for 72 h after ingestion of meal, and for this period, the participants were given an equally, strictly controlled diet low in As (13 μg tAs on day 0, 87 μg tAs on day 1 and 18 μg tAs on day 2, total 118 μg tAs days 0–2). Urinary tAs excreted in urine during 72 h after meal

were for the cod group, 561±87 μg tAs, the blue mussel group, 328±47 μg tAs, the salmon group, 161± 30 μg tAs and the control group, 49±13 μg tAs. Pending the results from the analysis, conclusions would be presented at the conference.

1.P30
Influence of probiotics preparations on an exchange of heavy metals

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We studied the action of probiotic preparations on the exchange of toxic elements in an organism of "Lomann-Braun" birds. During the 21 to 33 week of life, hens were fed a diet of cultured *Bacillus subtilis* at the rate of 10.0 ml/kg fodder (St. registration MH Russian Federation P no. 000792/01-2001 from 01.11.2001) and *Bifidobacterium longum*, in a dose of 0.7 ml/kg fodder (St. registration MH the Russian Federation nos. 77.99.11.3.U.5249.10.04 and 77.99.11.3.U.5246.10.04 with inclusion Federal register BAD). The action of probiotic preparations is defined by specific features of microorganisms. The estimation of biosubstrata (a fabric of a body, an egg) of birds on the maintenance of chemical elements was carried out by ISP-AES and ISP-MS. For the period of experience, fabrics of a body and an egg of *B. longum* was made in aggregate at a summer residence; the toxic elements (Pb, Cd, Ag, Sгб Al)—0.31 mmol/head, sporobacterin promoted a greater output with production—0.25 mmol/head, in a quantitative sense, were expressed in smaller contents. In the control group, *B. longum* promoted a decrease in the maintenance of fabrics in the body of the bird: silver 7.7% ($p<0{,}001$), strontium 22.7% ($p<0.05$), cadmium 31.7% ($p<0.01$), lead, 2.05 times ($p<0.001$). In the control group, when fed to hens, *B. subtilis* increased the levels of aluminium [3.3 times ($p<0{,}001$)], silver [38.5% ($p<0.01$)] and strontium (7.2%) in the body. The 7.3% increase in lead maintenance in the skilled group II, rather than the skilled group I, was statistically authentic. Research shows that probiotic preparations are capable of correcting mineral imbalance in species of animals; however, the given action is defined by specific features of microorganisms.

1.P31
Influence of the fermental preparation on the element status of carp in conditions of various nutrition security

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In conditions of pools investigated, the element statuses of carp ($n=30$, $m=20–30$) included in a diet of fermental preparation (Amylosubtilin) over a background of various maintenance of diets of starch are: 5–7; 12–14; 24–26; 36–38 and 48–50%. Changes in starch were accomplished through an additional introduction of wheat grain. The estimation of the element structure of the biosubstrata of a carp was made after 90 daily experiments, AEM and MSM. Use of fermental preparation Amylosubtilin G3x in feeding a carp is interfaced to selective change of structure of chemical elements in a body of a fish that is expressed in decrease in weight of chrome on 28–44% and increase in the maintenance of iodine—18–52, silicon—19–35%. Thus, the presence of a preparation in a diet is interfaced to increase the need of copper and cobalt in a fish, in conditions of decrease in need for zinc. Influence of a fermental preparation on the element status of a carp is interfaced to changes of specificity of interelement interactions in an organism that is expressed in more than three multiple decreases in number of authentic negative correlation communications between weights of separate elements. Thus, the most expressed action of Amylosubtilin G3x is its interaction of lead with exchanges of other elements, in particular, easing of the Pb–Ca antagonism. At the same time, displays of new interrelations of strontium with phosphorus ($r=0.97$), cobalt ($r=0.99$), manganese ($r=0.95$), and nickel ($r=0.97$) takes place.

1.P32
Mineral status of energy enterprise workers: results of correction

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Unfavorable factors of a technological character influence the element status of people who work at

unhealthy trade or live near industrial enterprises. So, we have studied the element status of 122 people, aged from 18 to 60, working at joint-stock company "Orenburg energy". Their industrial work record is from 5 to 30 years, and these people are in touch with unhealthy trade factors. We estimated their element status by studying chemical composition of their hair. We defined the content of macro- and trace elements in biosubstrate by atom and emission and mass spectronomy with inductively connected argon plasma. We used the method of system diagnostics for the correction of workers' element status. This method is based on using the preparations which have the bioelements (Bio-Zn, Bio-Mg, Bio-I, etc.) under individual prescription and in accordance with the results of the hair multielement analysis. The received data enables us to speak about positive effect of corrective measures regarding some chemical elements. Correction regarding Si (from 54±3.8 to 25.6±2.1 mg/kg, $p<0.05$) and As (from 0.06±0.006 to 0.05±0.005 mg/kg, $p<0.05$) index of exchange had the most evident influence. Fe (from 37.1±2.5 to 31.3±3.8 mg/kg, $p<0.05$) and Li (from 0.03±0.002 to 0.026±0.004 mg/kg, $p<0.05$) concentration reduced considerably. We have positively regarded this fact as an attempt to normalize the Fe content in an organism. Mg and P levels reduced and Ca level increased in hair, so we assume that mineral exchange can possibly be normalized as a result of a correction course. A tendency of K and Na to increase can probably be evident of metabolic processes and homeostasis damage (e.g., increase of insulin level in blood). Workers in fuel and energy complexes (FEC) are exposed to these factors. Hg (from 0.93±0.09 to 0.68±0.08 mg/kg, $p<0.05$) and Pb (from 1.40±0.41 to 1.30±0.35 mg/kg) level reduction is another positive normalizing effect.

1.P33

Trace element status of a Brazilian alternative food

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The Brazilian Satellite Centre to Trace Element-Institute for UNESCO was created in Brazil in 2004 focusing at the common needs of the countries in Latin America, the most unequal region in the world. Inequality and poverty has been, along decades, the region's main challenges. According to the World Bank, nearly 25% of the population of over 550 million inhabitants lives on less than 2 USD a day, suffering from hunger and malnutrition. Brazil is by far the largest country of Latin America, both in area and population, and the fifth with the highest population in the world (186,405,000). As good nutrition is an essential key for achieving the Millennium Development Goals for having good health and high quality of living, governmental and private sectors are finding ways to overcome the serious difficulties related to food security and malnutrition. The Brazilian Child Pastorate is an important nongovernmental organization devoted to health, nutrition, and children's education, since antenatal to 6 years of age. It operates in 33,000 communities distributed in 3,500 municipalities and rural areas serving over 2 million children and 150,000 pregnant women. Families learn how to prepare nutritious meals with low-cost ingredients locally available. The so-called multimistura is a low-cost, fast food preparation alternative satisfying local taste preferences, based on nonconventional ingredients/foods and/or agroindustrial by-products rich in different nutrients. This already successful approach, in Brazil, to fight malnutrition has been implemented in other 15 developing countries around the world. This study intended to evaluate the nutritional trace element status of the multimistura for which some components of this alternative food, namely, rice and wheat bran and flour and cassava leaves were analyzed by instrumental neutron activation analysis. The analytical data was statistically treated and the results discussed in terms of nutritional quality.

1.P34

Role of selenium physiology in prevention and treatment of mercury toxicity

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High-level mercury (Hg) exposure can harm fetal neurodevelopment, but consequences depend on molar ratios of Hg to selenium (Se). Se is required for enzyme (Se-enzyme) activities that protect against oxidative

damage in brain and other tissues. High-binding affinities between Hg and Se (10^{45}) result in sequestration of intracellular Se, preventing synthesis of Se-enzymes that are active in detoxification of oxygen radicals. Therefore, it is not surprising that oxidative damage is a major consequence of Hg toxicity. Supplemental Se replaces Se lost to Hg binding, thus maintaining Se-enzyme synthesis. Maternal consumption of foods that contain Hg in excess of Se is harmful, but consumption of similar amounts of Hg in foods with Se in molar excess of Hg has not been associated with harmful effects. Maternal consumption of pilot whale meat (Hg:Se ratio 4:1) harmed children in the Faroes, but eating ocean fish meats (average Hg:Se ratio 0.1:1) was not associated with adverse effects in the Seychelles, even though total Hg exposures were greater. Rat studies employing diets that reflect low to toxic methylmercury (MeHg) exposures were studied in the presence of dietary Se at low, normal, or rich levels. Resistance to MeHg toxicity was directly proportional to dietary Se. MeHg exposures that were lethal to rats fed low-Se diets were harmless to rats fed Se at levels slightly less than the average Se concentration present in ocean fish. These studies find blood Hg reflects MeHg exposure, but blood Hg:Se molar ratios provide better criteria for recognizing risk of MeHg toxicity (r^2=0.96, p<0.00001). Ocean fish are generally rich in Se, but availability of Se varies dramatically in fresh water fish Se. Thus, Hg levels that are safe in fresh water fish from areas with normal or rich Se may constitute Hg toxicity risks in areas where Se availability is poor. Poor Se availability may exacerbate Hg accumulation and toxicity in "Hg-hotspot" regions of the Northeastern U.S.

1.P35

Manganese status in free-ranging European bisons from Bialowieza primeval forest

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The manganese status in the liver, kidney, muscle, hair, and hoof of European bisons free-ranging in the Białowieża forest was determined (total of 120 samples). The material for analyses was obtained in the winter of 2002 from animals eliminated by annual culling. The material originated from 20 European bisons aged from 5 months to 5 years. Animals were grouped according to sex (males vs females) and age (calved up to 1 year of age vs animals aged over 2 years). The manganese content in the samples of tissues and organs (except hoof) was determined using the inductively coupled plasma-optical emission spectrometry method with the apparatus Jobin-Yvon/France, type I-Y 70+ in the accredited laboratory. In the hoof samples, the content of manganese was determined using the method inductively coupled plasma-mass spectrometry (ICP-MS) in the same laboratory. The obtained results were analyzed statistically with the programme Statistica™ 5.0 ANOVA module. No statistically significant differences in the manganese content were observed depending on the sex and age of animals. The mean manganese content in the tissues and organs of European bisons (n=20; mg/kg fresh tissue) was as follows: liver, 10±1.2; kidney, 4.5±1.2; rib, below detection limit (<1 ppm Mn); muscle, 2.3±0.4; hair, 15.2±4.3; hoof (dry matter), 3.0±2.2.

1.S01

Oral aluminum bioavailability from representative foods shows food provides much more Al to systemic circulation, and potential Al body burden, than does drinking water

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Oral Al bioavailability from water has been estimated, but Al bioavailability from food, which provides ~95% of daily Al intake for most people, has not been well described. The overall objective is to determine oral Al bioavailability from representative foods, compared to water. Foods containing the most Al are baking powder, creamers, salt, grain products, pizza cheese, and tea. Two foods containing an FDA-approved Al food additive, a vegetable (spinach), and tea beverage were compared to water. Processed cheese was prepared containing basic sodium Al phosphate (SALP) and the tracer 26Al (Nucl. Instr. Meth. Physics Res. B. 229:471–478, 2005). Male Fisher rats were trained to eat 1 g of cheese when they

had no stomach contents. They then consumed cheese containing 26Al-SALP (~1 to 2 nCi 26Al/g food), while receiving 27Al by i.v. infusion. Blood was withdrawn before and repeatedly up to 60 h after dosing for 26Al quantification by AMS and 27Al by ETAAS, to determine the area under the [Al]×time curve (AUC) of each. Oral 26Al bioavailability was determined as the sum of the trapezoidal (AUCpo/AUCiv)×(doseiv/dosepo). Oral Al bioavailability from cheese containing 1.5 or 3% basic 26Al-SALP was ~0.1 and 0.3%, respectively, which were significantly different. The C_{max} of Al was significantly greater than from water or biscuit containing acidic SALP, and the T_{max} was significantly greater than from biscuit containing acidic SALP, but significantly less than from water, using the same methods (Toxicology, 161:93–101, 2001 and 227,86–93, 2006). Considering (1) the relative oral bioavailability of Al from cheese containing basic SALP and biscuit containing 1 or 2% acidic 26Al-SALP (~0.11 and 0.13%), compared to water (~0.3%) and (2) the contribution of food and drinking water to the typical human's daily Al intake (~95 and 1.5%, respectively), these results suggest food provides much more Al that reaches systemic circulation and potentially target organs such as the brain, than does water (supported by NIH R01 ES11305).

1.S02

Boron in the environment and human population of San Juan, Argentina

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In 2005, water, river sediments, soils, aquatic plants, sweet corn, and human scalp hair samples were collected from the San José de Jachal region of San Juan, Argentina and boron levels determined by inductively coupled plasma mass spectrometry. Comparative data from a control population were obtained from the Rio Negro Valley region of northern Patagonia, Argentina. Filtered surface water samples (n=51) from the Rio Blanco, Presa Cuesta del Viento, Rio Jachal, Agua Negra, Rio Huaco, and irrigation channels of the agricultural La Pampa San José de Jachal region had boron levels ranging from 2.43 to 7.24 mg/l compared with Rio Negro (n=15) 0.06 to 0.14 mg/l. The corresponding river sediment levels were San José de Jachal (36.4 to 104.9 mg/kg B) and Rio Negro (0.48 to 2.45 mg/kg B). In San José de Jachal, the highest levels occurred in the upper Rio Blanco, the Rio Huaco, Agua Negra, and in the area of Niquivil (Rio Jachal). Boron concentrations in aquatic reed plant material ranged from 45.7 to 189.2 mg/kg (San José de Jachal) and 6.5 to 28.1 mg/kg (Rio Negro). Concentrations in surface soils ranged from 28 to 590 mg/kg (San José de Jachal) and 3.8 to 34.3 mg/kg B (Rio Negro). Corn leaf material from San José de Jachal (0.9 to 24.3 mg/kg B) showed considerable variability. Angualasto showed a difference between plants irrigated by water from the Rio Blanco (0.9 to 8.7 mg/kg) compared with ground water (0.15 to 0.72 mg/kg). Rio Negro values were 0.08 to 1.64 mg/kg B. The respective sweet corn kernel levels were 0.09 to 1.14 mg/kg (San José de Jachal) and 0.04 to 0.13 mg/kg B (Rio Negro). Finally, unwashed human scalp hair samples were collected from both regions. Sixteen individuals from Niquivil had a mean (SD) of 8.33 (3.54) mg/kb B. In Huaco, 13 cases had values of 7.78 (3.21) mg/kg B. Dependencies of the determined concentrations in each matrix on specific causal factors are discussed.

1.S03

Food-based strategies improve iron status in 12- to 20-month-old New Zealand children: a randomised controlled trial

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We evaluated the efficacy of an increased intake of red meat or the use of an iron-fortified milk for improving the iron status of healthy non-anaemic 12- to 20-month-old New Zealand children. In a 20-week partial double-blind randomised placebo-controlled intervention trial, 225 children were assigned to one of three groups: meat (n=90), fortified milk (n=45), or placebo (n=90). The meat group was encouraged to consume ~2.6 mg of iron from red meat dishes per day. The fortified milk and placebo groups replaced their regular milk with iron-fortified cow's milk (1.51 mg iron/100 g prepared milk) or non-fortified cow's milk (0.02 mg iron/100 g prepared milk), respectively. Non-fasting venipuncture blood samples

were collected at baseline and 20 weeks for haemoglobin, serum ferritin, serum transferrin receptor, and C-reactive protein determination. Over 20 weeks, adjusted geometric mean serum ferritin concentration increased by 44% (95% CI 14, 82%) in the fortified milk group (p=0.002), tended to decrease in the placebo group [14% decrease (95% CI −27, 1%); p=0.063], and did not change in the meat group (10% increase (95% CI −7, 30%); p=0.241). At 20 weeks, serum ferritin concentration was 68% (95% CI 27, 124%; p<0.001) greater in the fortified milk group than in the placebo group, and 29% (95% CI 2, 63%; p=0.033) greater in the meat group than in the placebo group. There were no intervention effects on haemoglobin or serum transferrin receptor concentration. Both food-based strategies are likely to prevent the decline in body iron stores that can occur during the second year of life. Only the fortified milk intervention increased iron stores, which is in line with the Australian and New Zealand Nutrient Reference Value requirement that iron stores should be increasing in this age group (supported by Health Research Council of New Zealand, Meat and Livestock Australia, Meat and Wool New Zealand, University of Otago).

1.S04
Bioavailability of selenium in Brazil nuts

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Higher than recommended dietary intakes of selenium (Se) may confer health benefits. Brazil nuts are a rich natural source of Se, yet its bioavailability from this source has not been investigated in humans. The aim was to assess the efficacy of Brazil nuts in increasing Se status, in comparison to selenomethionine and placebo. A semi-blinded, placebo-controlled trial was conducted with 56 healthy adults (18–60 years) with low Se status, who consumed two Brazil nuts containing an estimated 100 μg Se, 100 μg Se as selenomethionine (SeMet), or placebo daily for 12 weeks. Because of the variability in Se concentrations in Brazil nuts, the actual intake from nuts averaged 54 μg/day (possible range, 20–100 μg). Plasma Se, plasma and whole blood glutathione peroxidase (GPx) activities were determined in fasting blood samples at baseline, weeks 2, 4, 8, 12, and effects of treatments compared. Mean (SD) baseline plasma Se concentrations were 90 (13), 92 (14), 89 (14) μg/L in Brazil nut, SeMet and placebo groups, respectively. Plasma Se increased by 68, 73, 6.9%, whole blood GPx by 12.6, 6.4, 1.4%, and plasma GPx by −4.0, 12.1, 6.4% in the three groups. The change over time in plasma Se (p<0.0001) and plasma GPx activity (p<0.001) in Brazil nut and SeMet groups differed significantly from Placebo group, but not from each other (p=0.165). The change in whole blood GPx activity was greater in Brazil nut than placebo group (p<0.001) and SeMet groups, although the latter was not significant (p=0.087). Daily consumption of two Brazil nuts is at least as effective for increasing Se status and enhancing GPx activity, as 100 μg Se as selenomethionine, in spite of lower Se intake from nuts. Therefore, Se from Brazil nuts may be more bioavailable than selenomethionine. Brazil nuts are a convenient source of Se; consumption of this high-Se food would avoid the need for fortification or supplements to improve Se status of New Zealanders.

1.S05
Molybdenum content in infant formulas from North America

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Molybdenum (Mo) is an essential trace element that is required by xanthine oxidase, aldehyde oxidase and sulphite oxidase. The Mo content of human milk (HM) declines during the first 12 weeks of gestation from 5 to 1 μg/L. There is evidence that the content of Mo in HM may be regulated homeostatically. Presently, the Mo content of infant formulas (F) is not standardized by either statute or manufacturing practice. Therefore, our goal was to analyze formula reflecting manufacturing locations across Canada (Winnipeg) and the United States (Baltimore, Grand Forks, and Seattle). Powders were wet ashed using Parr Microwave Digestion bombs. Using furnace Varian AAS with certified reference milk powders (NIST whole milk (0.29±0.13 μg/L) and NIST nonfat milk powders (0.34 μg/L) falling in the normal range, our analysis of F from Canada indicated Mo concentrations [μg/L, mean+SD (range)] of 36+14 (17–64);

Baltimore, 43+14 (20–67); Grand Forks, 25+17 (7–76); Seattle (in progress) vs 5 (2–23) in HM; Mo is a natural constituent of protein such that variable concentrations among F probably reflected differences in protein sources. The AI reflects Mo intake of infants fed HM and is: 0–6 months, 2 μg/day, (0.3 μg/kg/day); 7–12 months, 3 μg/day, (0.3 μg/kg/d). Mo UL for infants is not determinable because of insufficient data, although concern has been expressed about the infant's ability to handle excess MO. Because our findings indicate higher concentrations of Mo in F than in HM, further research is needed to determine whether or not the forms of Mo are of comparable bioavailability and whether higher content is an issue of concern (supported by Canadian Institutes of Health Research and the Manitoba Institute of Child Health).

1.S06

Trace metal abundance soil and grass in Iceland: links with sheep scrapie

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Recurrent outbreaks of scrapie in Iceland occur despite intense study into this incurable and fatal prion disease. There are many, often disputed, theories as to the cause of scrapie even though understanding of prion biology is increasing. Trace metals have an important role in the biology of prion disease, and a possible link with levels of minerals, such as Cu, Mn and Zn, in the farm environment is tantalising. This study found that the pH of Icelandic soils was significantly higher on scrapie-prone farms. Conversely, soil OM was significantly lower on scrapie-prone farms. It was found that trace element concentrations differed between the categories of farms, with total soil Cu, Mn and Zn higher, and total S lower, on scrapie-prone Icelandic farms. These properties may have an impact on the health of sheep on these farms, through either increases to the trace metal content of dietary herbage or increased intake of metals through inadvertent soil ingestion. It was demonstrated that other (non-scrapie) factors have significant influence on the trace metal content of Icelandic soils. Levels of OM and pH of soils showed significant correlation with the trace metal content of soils from Icelandic farms. The pH and total Fe content of cultivated soils were significantly lower than those from uncultivated pastures in Iceland. Conversely, OM and total S concentrations were significantly higher in cultivated soils. In addition, total soil S levels were significantly higher from wetland habitats: demonstrating the complex nature of soil chemistry and highlighting the difficulty of assigning causal factors to variations in trace metal abundance on scrapie-prone Icelandic farms. Correlation analysis identified high soil pH and low OM content as major factors influencing the low Zn content of Icelandic grass from scrapie-prone farms. These two parameters could therefore be considered risk factors for scrapie.

1.S07

Bioaccessibility of zinc in whole eggs by continuous-flow dialysis method

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Zinc is an essential trace element, which has recently received increasing attention among other micronutrients because of their importance in child growth and development and in the immune system. Zinc is widely available in various foods, but its bioavailability is highly variable. The good absorbable zinc sources are animal products which may be too expensive or not available for some groups of population. Therefore, hen's egg, an inexpensive common food with concentrated nutrients, could be a potential food source to improve zinc nutrition. Increasing zinc content in eggs was conducted by fortifying laying hen diets using both inorganic (zinc sulfate) and organic forms (zinc amino acid chelate). Fortifying levels of zinc from 300 to 1,800 μg/g in the diet produced eggs with suboptimal and optimal levels of zinc content, about 15 and 18 μg/g. In this study, the bioavailability of zinc in the fortified eggs at suboptimal and optimal levels were evaluated by continuous-flow dialysis method. It was found that the dialyzed zinc in the zinc amino acid chelate eggs, which contained 14.8 and 17.3 μg Zn/g, were 79.1 and 78.5% of total zinc, respectively. They were slightly higher than the levels of the zinc sulfate-

fortified eggs, 71.8 and 74.1% of total zinc, respectively, which contained 15.0 and 18.2 μg Zn/g. The zinc bioavailability of the controlled eggs (without zinc fortification) containing 11.5 μg Zn/g was 83.6% of total zinc. Based on the actual values of total zinc content in the eggs and their bioavailabilities, the available zinc in the studied eggs were not significantly different at the same levels of fortified zinc regardless of their chemical forms. They were 11.7 and 10.8 μg/g for suboptimal levels and 13.6 and 13.4 μg/g of optimal levels of zinc in the fortified eggs. Therefore, the fortified eggs with both organic and inorganic forms with the highest available zinc were selected for further study.

1.S08

Analytical data on Iranian typical diets and its bioavailability aspect by trace elemental study in daily diets

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In this article, some of the main aspects of a comprehensive study on trace elemental content of a few typical daily diets of Iranians are discussed. The interrelation of 137Cs and a few more similar and radiological functioning elements such as Sr, Rb, I, K, Mg, and Ca with themselves and others (e.g., essentials and toxicologicals) are discussed. A part of this work is about the measurement of the natural relevant elements to their appropriate radioelements measured in daily diets of a few study groups. Their likely interrelation and interaction among their own or with other groups of trace elements are to be discussed based on data obtained via daily diets of the aforementioned trace elements. Nevertheless, the protection role of these trace metals in human organs and the general interrelation among the trace elements are the main issue of this paper. The matter regarding the origin of natural cesium and its radioactive fall out is to be discussed. The effect of 137Cs and other radionuclides of the foodstuff depend upon direct exposure or via the mineral and organic species of radionuclides in the soil and environment to be transferred to nutritive species which are consumed. In this paper, the toxic trace elements and their interrelation with nutritional/essential and radiological elements were taken into consideration. However, the actual study groups are Iranian, and their daily diets were prepared through dietary recording and duplicate portion methods.

1.S09

Investigation of chromium, copper, cobalt, and molybdenum in some cereals consumed in Kenya

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A review was conducted on the role of cobalt, chromium, copper and molybdenum in human health. A study was carried out to determine the levels of these elements in rice, maize and wheat flour consumed in Kenya. Dry ashing and acid solubilization procedures were used to prepare the samples. The samples were analysed using Atomic Absorption Spectrophotometer (Model, Chemtech Analytical 2000). Chromium in the samples had a range of 0.5 to 2.0 mg/kg with a mean of 1.04, while copper ranged between 1.50 and 9.72 mg/kg with a mean of 3.74. Molybdenum was not detected in any of the samples. Cobalt was only detected in wheat flour (mean of 6.44 mg/kg). These values were compared with the recommended daily allowances. Disclosure: The author is a member of Trace Element Institute for UNESCO—Kenya Satelite Center which is affiliated to Trace Element Institute for UNESCO (Lyon).

1.S10

The dietary boron intake in China

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Boron is a trace mineral that is essential for plants. Boron may also be essential for humans and animals. In 1999, dietary boron intake was researched in some countries; range is 1.1–2.1 mg/day for males, but these were not Asian and Chinese dietary intakes data. Therefore, existing boron intake data may not be representative if there are no boron dietary intake data from China (the largest developing country in the world) to set up global RNI standard. For this reason, the dietary intake of boron has been obtained in

Chinese adult males using the 2000 Chinese Total Diet Study approach. Average dietary intake of boron in 12 Chinese provinces was 1.384 mg/day, range 0.891 –1.976 mg/day. Average dietary intakes of boron for adult males in Heilongjiang, Liaoning, Hebei, Henan, Shaanxi, Ningxia, Shanghai, Fujian, Jiangxi, Hubei, Sichuan, and Guangxi provinces or city, respectively, were 0.891, 1.531, 1.565, 1.976, 1.237, 1.630, 1.433, 1.117, 0.969, 1.314, 1.228, and 1.720 mg/day. Because available boron level from soil in most parts of China is low, ranging from 0.25 to 0.50 mg/kg, the dietary boron intake of China is not higher than that of other countries. The main food sources of boron in the Chinese diet were vegetables (32.2%), cereals (31.7%), fruits (14.8%), and legumes (13.2%).

1.S11

Women's zinc absorption is unaffected by dietary calcium, with or without high-phytate foods

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While some have hypothesized that calcium increases the inhibitory effect of phytic acid on zinc absorption by forming insoluble Ca–Zn–phytate complexes, others have suggested that calcium may competitively bind phytic acid, reducing the phytic acid inhibition of zinc absorption. We tested the influence of approximately 700 (LCa) or 1,900 (HCa) mg/day dietary calcium with 440 (LP) or 1,800 (HP) mg/day phytic acid on zinc absorption in a 2×2 factorial design. Minerals were measured by ICAP and phytic acid by the AOAC extraction and ion-exchange method. The weighed diets, consisting of ordinary foods, contained ~11.5 mg Zn/day, with a phytate:Zn molar ratio of ~4 for LP and 15 for HP. Zinc absorption from each of the four 1-day menus was determined with ten healthy women by extrinsically labeling the foods with 65Zn and measuring retention by whole body scintillation counting. For each absorption measurement, individual differences in endogenous Zn excretion were corrected for by extrapolating back to baseline along the linear portion of a 4-week semilogarithmic retention curve. Data were analyzed by ANOVA with Tukey's pair-wise contrasts. Zinc absorption from the LCaLP, LCaHP, HCaLP, and HCaHP diets, were 33, 27, 39, and 26% (pooled SD=7.6), or 3.8, 3.0, 4.5, and 3.2 mg/day (pooled SD=0.28), respectively. Phytic acid significantly decreased zinc absorption ($p<0.001$), but calcium did not, nor did calcium significantly interact with phytic acid. We conclude that calcium in the range normally consumed, even with calcium- fortified diets, does not interfere with Zn absorption.

Category 2: Cancer

2.P01

Treatment of acute lymphocytic leukemia using zinc adjuvant with chemotherapy and radiation—a case history and hypothesis

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Low blood levels of zinc are often noted in acute lymphocytic leukemia (ALL), but zinc is not administered as part of any modern chemotherapy program in the treatment of ALL. Upon noting low blood levels of zinc in a 3-year-old 11.3 kg girl, zinc at the rate of 3.18 mg/kg body weight/day was administered from the start of chemotherapy through the full 3 years of maintenance therapy. Dosage was split with 18 mg given at breakfast and 18 mg zinc with supper. The result was a bone marrow remission from 95+% blast cells to an observed zero blast cell count in both hips within the first 14 days of treatment which never relapsed. In addition to the reduction of blast cells to an observed count of zero (not a single leukemic or normal blast), red blood cell production and other hemopoietic functions returned to normal at a clinically remarkable rate. There were no side effects from zinc or chemotherapy at any time, and zinc is hypothesized to have improved the patient's overall ability to withstand toxic effects of chemotherapy. This report identifies zinc treatment as being vital to rapid and permanent recovery from ALL. The extremely broad role of zinc in pre-leukemic adverse health conditions, viral, fungal and tumoral immunity, hemopoietics, cell growth, division and differentiation, genetics, and chemotherapy interactions are considered. Identical results also occurred in 13 other children with ALL whose parents chose to treat with zinc adjuvant. As treatment with zinc and other identified deficient nutrients, particularly magnesium,

did not appear injurious in ALL, and they appear to be highly beneficial, controlled clinical studies of zinc (3.18 mg/kg body weight/day) with magnesium (8.0 mg/kg body weight/day) as adjuvants to chemotherapy in the treatment of childhood ALL are suggested. Treatment with zinc adjuvant is hypothesized to accelerate recovery from ALL, and in conjunction with chemotherapy, cure ALL.

2.P02

Effect of selenium supplementation on the serum level of prostate-specific antigen (PSA) in high PSA subjects

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Prostate-specific antigen (PSA) is widely used clinically for prostate cancer diagnostics and as an indicator of therapeutic efficacy. In vitro studies showed that selenium inhibits human prostate cancer cell growth and reduces androgen signaling and androgen receptor (AR)-mediated gene expression, including prostate-specific antigen (PSA), in human prostate cancer cells. To assess the effect of selenium supplementation on the changes of serum levels of PSA, four high PSA subjects and six low PSA healthy controls were received a daily supplement with 200 mcg of selenium as a form of sodium selenite. The subjects were recruited at the Department of Public Health, Gunma University Graduate School of Medicine. Inclusion criteria for the high PSA subjects were PSA>3.0 for 50–64 years, 3.5 for 65–69 years, and 4.0 ng/ml for 70 years and more and histological or ultrasonographically free of prostate cancer. Serum levels of PSA were assessed at baseline and after 1, 3, (4), and 6 months by immunometry. The protocol was approved by the ethics committee of Gunma University School of Medicine. The study is now still ongoing, however, we can see the tendency of decreasing of serum PSA levels in the high PSA subjects and no change, at least not increasing, in serum PSA levels of the low PSA healthy controls.

2.P03

Selected trace elements and cadmium concentrations in malignant, hypertrophic, and normal prostatic tissues

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The aim of the study was to determine Cd, Se, Zn, Ca, Mg, and Cu concentrations in prostatic tissues: post mortem, hypertrophic, and neoplastic. 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 examined pathomorphologically to confirm the diagnosis. Zn, Cu, Ca, and Mg concentrations were determined with the flame and Cd with flameless AAS method, whereas Se with the spectrofluorimetric method. We have now extend our study to include the determination of insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) in blood plasma. Based on our investigations, cadmium concentration was found to be decreased in about 22% of patients with prostatic hypertrophy and more than twice in those with prostatic cancer compared to the post mortem group. The highest Zn level was demonstrated in the subjects with prostatic hypertrophy (over 100% increase in relation to the post mortem group), whereas in the group with the diagnosed neoplasm, it was lower by almost 50%. Higher Mg and Ca levels were found in hypertrophic and lower in cancerous tissues, like in the case of zinc. Copper and selenium concentrations were found to be higher in both patients with prostatic hypertrophy and those with neoplasm compared to the post mortem group. Also, our research showed positive correlation between zinc and copper (r= 0.80) in the post mortem tissue; in the remaining tissues, the correlation between these elements was significantly lower. The study was supported by the Grant Ministry of Science and Higher Education (no. N405 015 31/0783).

2.P04

Risk analysis of selenium in Korean health-functional foods

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Selenium (Se) is an essential nutrient for the health of humans and animals, but excessive Se intake can bring about selenosis, which leads to hair or nail loss, and even a morphological change of the nail. To determine the dietary risk of selenium exposure via food ingestion, samples of health-functional foods containing selenium were collected from food markets in Korea, and the concentration of selenium was found to range from 0.1 to 0.8 ppm. Intake data for agricultural foods or processed foods were taken from the Korea National Health Examination and Nutrition Survey (KNHANES) and the nation's sources of daily recommended doses, respectively. Human epidemiological data studied by Yang et al. were used as a reliable toxic endpoint (0.015 mg/kg/day, NOAEL), and the uncertainty factor f 3 was then applied for interindividual variability. As a result, the acceptable daily intake (ADI) of 5 μg/kg body weight for Se could be derived. From this study, selenium would not be harmful to Korean people as long as they ingested the functional foods with the average daily intake of 0.002 to 1.407 μg/kg body weight. Even after taking into consideration the highest intake of 1.438 to 4.345 μg/kg body weight resulting from a maximal supplement intake plus other additional sources of food intake, the hazard index (HI), which can be calculated by dividing the ratio of selenium concentration by its reference concentration, did not exceed one. The findings of this study suggest that there are not any groups of individuals with a high susceptibility to compromised health. However, if any individual ingested multiple sources of foods containing Se simultaneously, his or her daily intake of selenium is thereby increased to 5.463 μg/kg body weight, which exceeds the safety level. Therefore, the implications of the findings of this risk assessment are such that an individual should not consume multiple sources of selenium-containing foods concurrently but rather one at a time.

2.P05

Design, synthesis, and investigation of copper (II) complex of 3-aminoflavone (af) with anticancer activity

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Flavanoids have attracted attention for a variety of biological activity including antitumor, antioxidative, antiviral, antibacterial, and antimutagenic. The novel potential anticancer agent *cis*-Pt(II) complex of 3-aminoflavone, i.e., *cis*-[Pt(af)2Cl2] structurally related to cisplatin was found to prolong the survival time of mice with lymphoid leukemia. Nowadays, studies are focused in searching for novel potential antitumor metal complexes with high anticancer activity, broader range of action, and less toxic than cisplatin. As metal ions may be involved in the biological effect of the flavanoid system, we synthesized a number of new coordination compounds with af ligand. To investigate the binding in detail, a crystal structure of a representative compound, viz [Cu(af)2(NO3)2] has been determined. The pronounced antiproliferative activity of the compound was assessed using cultured L 1210 cells and was compared to the reference compound cisplatin. It may be a promising future anticancer drug. Financial support by the Medical University of Lodz (grant no. 502-13-339 and grant no. 503-3016-2) is gratefully acknowledged.

2.P06

Effect of fluoxetine on tolerance to analgesic effect of morphine in mice with skin cancer

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Adjuvant drugs that can attenuate or inhibit the development of tolerance to morphine may lead to improved management of pain in chronic disease such as cancer. Recently, we have shown that 5-HT1A receptors of dorsal raphe magnus are involved in tolerance to morphine analgesia. The aim of this study

is to investigate the effect of fluoxetine, as a specific 5-HT reuptake inhibitor, on tolerance induced to morphine analgesic effect in an animal model of skin cancer. A study was carried on female albino Swiss mice with skin cancer weighing 22–25 g. Animals were kept in 12 h light/dark cycle and had access to food freely. For skin tumorigenesis, mice were given Iron-dextran (1 mg/mice for 15 days), initiated with single dose of 7,12-dimethylbenz(a)anthracene (DMBA) and promoted by multiple dose of croton oil. Tolerance to thermal acute pain was induced by daily s.c. injection of morphine (5 mg/kg for 30 days) and using hot plate method. Results obtained from this study show that pain threshold in mice with skin cancer is significantly low than normal mice. In those mice which were co-treated daily with morphine (5 mg/kg, s.c.) and fluoxetine (0.16, 0.32, or 0.64 mg/kg, i.p.), delay in development of tolerance was observed by fluoxetine at the dose of 0.64 mg/kg. Fluoxetine (0.64 mg/kg, i.p.) attenuated the development of tolerance from day 20, for control group, to day 25. We offer that the concurrent use of morphine with fluoxetine may produce good cancer pain control and attenuate development of tolerance.

2.P07

Inhibitory effect of *Chrozophora tinctora* extract (methanol) on ferric nitrilotriacetate-induced oxidative stress in rat kidney tissue

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Intraperitoneal injection of ferric nitrilotriacetate (Fe-NTA) to laboratory animals lead to oxidative stress, lipid peroxidation (Lpo), and tissue injuries. However, our recent report indicates that *Chrozophora tinctoria* (*C. tinctoria*) can inhibit oxidative stress and delays tumor incidences in mice skin. In this study, we attempt to extract and evaluate the inhibitory effect of *C. tinctoria* on Fe-NTA-induced oxidative stress in rat kidney tissue. To analyze methanol extract of *C. tinctoria*, the reverse-phase HPLC technique was used. Male Spraque–Dawley rats were given orally different doses of *C. tinctoria* (10, 20, 30 mg /kg) before and after Fe-NTA (9 mg Fe/kg) treatment. Also, groups of animals received either *C. tinctoria* or Fe-NTA alone. All the animals were killed, and the kidneys were taken out. The biochemical estimation such as lipid peroxidation and [3H]thymidine uptake were carried out on the kidney tissue. The obtained results indicate that *C. tinctoria* at the dose of 30 mg/kg inhibits the Lpo induction and DNA synthesis induced by Fe-NTA. Our data indicate that *C. tinctoria* is more effective before Fe-NTA treatment. The results indicate that the inhibitory effect of *C. tinctoria* may act through different mechanisms. Involvement of *C. tinctoria* in the diminishment of LPO and [3H] thymidine uptake indicates that *C. tinctoria* may play an important role in scavenging the harmful free radicals in rat kidney tissue.

2.P08

Clinical elementology in oncology: experiences and proposals from Germany

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Trace elements are of growing importance for clinical oncological disciplines. Nevertheless, they have been broadly neglected so far. As indicated by Zaichik and Pollet (TEM 2007), a new scientific discipline of "medical elementology" should be established: "We will hope that the new century, that has just begun, will be the century of medical elementology". With this background, our working group AKTE has set itself the task of scientifically acquiring the significance of trace elements in oncology. This should be the base of "Clinical Elementology", which can be an important subdiscipline of "Medical Elementolgy". In the last 5 years, AKTE performed different preclinical and clinical studies mainly on selenium, showing distinctive antitumorous, antiedematous, and radioprotective features. In particular, a prospective ran-

domized observation study evaluated, whether sodium selenite is able to compensate selenium deficiency and prevent radiation-induced diarrhea in pelvic radiotherapy. There has been a statistically significant difference between the study groups towards a lower incidence of diarrhea in week 5 with selenium supplementation. With a median follow-up of 24 months, the actuarial 5-year overall survival rate of patients with supplementation of selenium was 92.4% comparing to the control group with 83.3%. The positive results of the studies lead us to the conclusion that the science of trace elements have to be integrated in the oncological sciences and the education and training in theoretical and clinical oncological disciplines. It is our aim to develop and establish a curriculum for the continuous medical education for physicians. So, we like to conclude with the wish that the coming century will not be only the century of medical elementology, but also of clinical elementology.

2.P09

Effects of long-term antioxidant (selenium and vitamin e) and ibuprofen antioxidant therapy on oxidative stress and lipid peroxidation

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Oxidative stress is caused by an imbalance between production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Antioxidants in biological systems have multiple purposes, including defending against oxidative damage and participating in the major signalling pathways of the cells. One major action of antioxidants in cells is to prevent damage due to the action of reactive oxygen species. In complex interactions with vitamin E and polyunsaturated fatty acids, selenium plays an essential biological role as part of the enzyme GSH-Px, which protects tissues against peroxidative damage by catalyzing the reduction of lipid hydrogen peroxide or organic peroxides. Ibuprofen is a NSAID used for relief of symptoms of several diseases and as analgesic, especially where there is an inflammatory component. Selenium, vitamin E, ibuprofen, and placebo were used for long-term therapy for 80 patients with reduced antioxidative defense system action. We analyzed various lipid peroxidation parameters (MDA, TAS, NEFA, GSH, plasma chemiluminescence, etc.) during and after 1-year supplementation. Results indicate that long-term usage of antioxidants and ibuprofen increased the amount of Se and vit E in the patient's plasma thereby significantly preventing lipid peroxidation. Our thanks to "PharmaNord" (Denmark), "Olainfarm" (Latvia) for medicines, and Randox Laboratories Ltd. (Crumlin, UK) for technical support.

2.P10

Trace elements levels and cancer incidence in Europe

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Differences between cancer rates and diet are apparent if data from WHO/IARC cancer incidence data and FAO data on diet are compared for different countries. There is, for example, a clear positive correlation between the intake of red meat and colon cancer incidence and between dietary animal fat and breast cancer rates. These relationships have been confirmed and explained by more detailed laboratory and epidemiological studies and are of value in developing cancer prevention strategies. There are many suggestions in the scientific and medical literature of associations between trace element levels and the incidence of different types of cancer. High levels of arsenic, for example, have been linked to increased rates of skin and bladder cancer, and cadmium has been suggested to have estrogenic properties and to be implicated in breast and prostate cancer. Selenium and iodine, on the other hand, have been suggested to be generally protective against cancer. In this study, the age standardized incidence rates of cancer from the IARC/WHO database for 2002 are compared with the level of a number of trace elements across Europe using data from the new Forum of

European Geological Surveys (FOREGS) database. The data show some positive correlations, for example, between the highest levels of cadmium and breast and prostate cancer incidence, although no relationship is identified between low levels of selenium and any of the types of cancer studied. Relationships between several types of cancer and the levels of arsenic, nickel, and uranium, and of selenium and iodine in the surface environment of Europe are also presented.

2.P11

Trace elements that protect against the free radical: factor analysis

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The aim of this study was to study relationship between the whole blood bioelement multielement profile (MP) and sister chromatide exchange (SCE) in the peripheral blood lymphocytes as an indicator of the radical exposure. Ten apparently healthy registered nurses participated in this randomized double-blind study at the time of their annual health checkup. The study was conducted by following the principles of Helsinki declaration and approved by the IMI Ethical Committee. We used ICP MS for the MP TE whole-blood analysis at the Center for Biotic Medicine, Moscow, Russia; SCE was analyzed by conventional light microscopy at the Laboratory for Mutagenesis, IMI. The data were subjected to the principal component analysis using the SAS statistical package. The MP TE of 41 elements was separated into seven principal components, whereas 9 element components were extracted (Ag, Au, Bi, Co, Ga, Ge, Hg, Sb, Sn). The remaining 32 elements comprised seven components: the second largest component (Factor 2, F2) with Eigenvalue of 18,9 showed that Se, Sr, Mn, Cu, and Be are inversely related to the SCE. The first (F1): Mg, Fe, Zn, P, K, (−Pt), (−I), (-Cd), Si, Rb; the third (F3): Mo, Al, W, Ni, Ca; the fourth (F4): La, Na, Ba, (−Pb), Li; the fifth (F5): V, Cr; the sixth (F6): Tl, B; the seventh (F7): Zr. This is the first clear cut evidence that the free radical oxidative damage is inversely related to the TE nutritional status. This finding emphasized the essential role of adequate TE nutrition for the health protection under the normal everyday living and working conditions especially that of Se, Sr, Mn, and Cu which have, respectively, proven cancer protection qualities, a role in the bone metabolism, co-involvement with the Fe metabolism, and the quality of the blood vessel walls.

2.P12

Investigation of cytotoxic effects of thallium acetate and ellagic acid on c6 cell line

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The aim of the present study was to investigate the cytotoxic dose–response relationships of the trace element thallium and ellagic acid investigated on C6 cell line. C6 cell line was incubated with different concentrations of thallium acetate and ellagic acid. Cell viability was determined by MTT and Neutral Red assays. Ellagic acid and thallium were tested in the concentration range of 1 to 1,000 μM (1, 10, 25, 50, 100, 250, 500, 750, 1,000 μM). Cell proliferation was measured after 24, 48, 72, and 96 h using the MTT and NR assays. The results showed that thallium had cytotoxic effects and decrease the cell viability in C6 cell line when compared to ellagic acid's cytotoxic effects. According to these results, ellagic acid had a concentration of 100 μM dose 31%, 500 μM dose 13% of C6. Thallium was found to be less cytotoxic than ellagic acid, at a concentration of 500 μM. After 48-h incubation; thallium at concentration of 500 μM caused decrease of C6 cell viability 500 μM 47%, respectively. According to these results, 500μM concentration of ellagic acid showed more cytotoxic effect than thallium.

2.P13

Association of dietary magnesium and DNA repair capacity with lung cancer risk

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Magnesium is an essential nutrient for humans because it is required for the maintenance of genomic

stability and DNA repair. Because the associations between dietary magnesium (Mg) intake and lung cancer risk have not been reported, we examined the relationship of Mg intake and DNA repair capacity (DRC) on lung cancer risk in an ongoing case-control study. A total of 1,139 incident lung cancer cases and 1,210 matched healthy controls with data on DRC were used to achieve our objective. A modified National Cancer Institute-Block Food Frequency Questionnaire and a lifestyle questionnaire were interviewer-administered to each participant. Cellular DRC was assessed by the host-cell reactivation assay, which measured nucleotide excision repair capacity in peripheral blood lymphocyte cultures. Mg intake in our control population was comparable to Mg intake in the National Health and Nutrition Examination Survey (1999–2000). After adjustment for recognized confounding factors, the odds ratios (OR) and 95% confidence intervals (CI) of lung cancer according to increasing quartiles of dietary magnesium intake for all subjects were: 1.0, 0.8 (0.7–1.1), 0.6 (0.5–0.8), 0.5 (0.4–0.6), respectively (p trend<0.0001). Similar ORs and trends were observed in men and women. In stratified analysis, using those with high dietary Mg and proficient DRC as the referent group, the OR for all subjects were: 1.0, 1.4 (1.0–1.8) for high dietary Mg and suboptimal DRC, 1.5 (1.2–2.0) for low dietary Mg and proficient DRC, and 2.4 (1.8–3.0) for low dietary Mg and suboptimal DRC, respectively (p trend<0.0001). These associations were generally similar in men and women. Our results suggest that low dietary Mg intake was associated with increased risk of lung cancer, and there may be joint effects between Mg intake and DRC on lung cancer risk.

2.P14

Trace elemental constitution of patients with oral submucous fibrosis and leukoplakia and its possible correlation with endogenous antioxidant status

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Oral submucous fibrosis (OSF) and leukoplakia are two critical types of precancerous conditions affecting oral mucosal surface that could subsequently develop into oral cancer. Oral cancer prevalence ranks first among men and third among women in India. As macro- and microelements are known to play important role in metabolic pathways, elemental status often indicates a reflection of general health profile of a subject. Recent research has shown variation in elemental concentration in tissues during growth of tumors and also in subjects suffering from various cancer types. In this perspective, the present work aims to evaluate serum trace elements (TE) of OSF and leukoplakia patients to assess the status of these two precancerous conditions in terms of progression towards cancerous growth. Furthermore, the work also focuses to correlate TE profile with the endogenous antioxidant activity considering the enzyme catalase (CAT), which indicates the stress developed during any pathological conditions. TE profile assessed employing energy dispersive X-ray fluorescence (EDXRF) technique reflected significant alteration in levels of Ca, Cu, Zn, Se, Co, and Cr in OSF and leukoplakia patients when compared to that of the control group. Sr and Rb concentrations also revealed an interesting change. While Ca and Se concentrations revealed an increasing trend in both the premalignant conditions, levels of Cu, Zn, Co, Cr, Sr, and Rb showed a reverse pattern in comparison to that of the control group. Significant decrease in CAT activity in both types of patients reflected depletion in antioxidant status. The work stands out to be a signal to utilize TE profile assessment in these premalignant diseases, which could be used as prophylactic measure for preventing progression of these precancerous conditions towards malignancy.

2.P15

Characterization of trace elements and metalloenzymes and its association with the immune status in oral cancer patients

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Production of excessive free radicals has been implicated in the pathogenesis of many diseases including neoplastic growth. Essential elements like copper, zinc, and selenium have been shown to be involved in protection against oxidative stress in cells. Plasma trace elements (selenium, zinc, and copper) and RBC antioxidant metalloenzymes (glutathione peroxidase and superoxide dismutase) were studied in 45 patients suffering from oral cancer (OC). A control group of 84 healthy subjects was selected. Trace element analysis was done employing the EDXRF technique. There was a significant fall in the mean values of copper, zinc, selenium levels, and depletion in activities of superoxide dismutase and glutathione peroxidase in OC patients when compared to the control subjects. Data indicate positive correlation between levels of zinc and copper with superoxide dismutase activity, while selenium concentration reveals such correlation with activity of glutathione peroxidase. The observed susceptibility of the patients with common infections is reflected in the immune response of the subjects assessed through analysis of interleukin production. Depletion of important micronutrients, especially zinc, in OC patients is corroborated by the observed deregulation in enzyme activity and impairment of immune response of the subjects.

2.P16

Investigation of cytotoxic effects of the boron compounds on leukemia cell line

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Recently, as the effects of malignancies have come out, the attention has been paid to various boron compounds. There are many experiments in which boron compounds work as a proteozom inhibitor on cancer cells. In our study, we searched out the effects of boric acid and sodium tetra borate on the acute leukemia cell lineage. We evaluated the cytotoxic effect by the help of MTT and Neutral Red method by following the overnight incubation of leukemic cells in 100, 250, 500, 1,000 μl concentration of boric acid and sodium tetraborate. We found that at the boric acid concentration of 500 μl, there are dead cells at the rate of 50%, at 1,000 μl at the rate of 70%, and for sodium tetra borate, a rate of 40%. On the other hand, in healthy human lymphocytes used as a control, this rate did not reach 30% even in the highest concentration. We detected an increase of 20% in both compounds in the same concentrations and in the lysosomal activity. We evaluated the apoptotic effect of boric acid and sodium tetraborate on the lymphocyte and leukemic cell lines in the flow cytometry using acridine orange methods An apoptotic effect was found at a 1,000-μl concentration 2.5% in normal lymphocytes, and at 1,000 μl concentration, 8.8% HL-60 cell lines, respectively. We examined the boron compounds by means of the transmission electron microscope to detect their effects on the acute leukemia cells. We observed that boric acid at a 500-μl dose caused double nucleus and micronucleus formation in both acute leukemia cells and lymphocytes. We determined that an expansion in the dimension of mitochondrium and a deformation in cristas appeared. We found that boric acid was more effective than sodium tetraborate on the leukemia cell lines. According to our results, particularly boric acid showed a toxic effect on HL-60 (acute leukemia cells) in comparison to normal lymphocytes, and they also affected the mitochondrial pathway.

2.P17

Selenium supplementation in head and neck surgery

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We have recently reported about the positive influence of selenium substitution on the regression of interstitial lymph edema due to radiotherapy in head and neck cancer patients. The presented study was performed to evaluate the effects of selenium substitution during surgical procedures in the oral cavity and the neck tissue. We included 25 patients (5 women, 20 men) in the study, which was performed according the principles of GCP (Declaration of Helsinki). All patients suffered from histological confirmed squamous cell carcinoma of the mouth (n=10) or pharynx (n=15). We performed laser surgical resection in all patients and neck dissection in 20 of 25. Ten patients received neck dissection of

both sides. At the evening of surgical procedures, we measured the possibility of the patient to open the mouth (evaluation of edema in the oral cavity) and the amount of the neck (evaluation of the edema of the soft tissue). After the first measurements, the patients received 1,000 μg sodium selenite orally twice a day. The measurements were repeated every 24 h. We have observed a significant reduction of opening the mouth (p=0.03) within 24 h after selenium supplementation. The reduction of edema in the neck soft tissues becomes significant after 48 h. The normal nutrition was sufficient within 24 h after surgical procedures. The positive influence on the postoperative morbidity will be discussed in the presentation. The presented phase-II data show similar experiences in surgical oncology than in the postradiation edema. A larger study is planned to compare the observed results with the normal postoperative regression of edema.

2.P18

Boric acid and phenyl boric acid cytotoxicity in breast cancer cell lines

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Previously, we have reported that boric acid inhibits growth of prostate cancer cell lines. The mechanism by which boron causes inhibition in prostate cancer cell growth is still unknown. However, these positive findings encouraged our efforts to determine if boric acid has a similar effect on breast cancer cells. We have reported that addition of 1 mM boric acid (BA) to culture media is able to partially inhibit growth in some human breast cancer cell lines: ZR-75-1 and SK-BR-3 but not others: MCF-7, T47D, MDA-MB-231, and MDA-MB-435. In this study, we report that phenyl boric acid (PBA), a structurally bulkier analog of BA, was able to partially inhibit both ZR-75-1 and MCF-7. After treatment with BA, we performed DNA analysis on both the floating and attached ZR-75-1 cells. DNA analysis of these two cell populations indicated that attached cells were actively growing, while floating cells were undergoing apoptosis in a dose-dependent manner. To confirm these results, ZR-75-1 cells were analyzed by fluorescent microscopy staining and were shown to be undergoing cell death by apoptosis and not necrosis. After treatment with PBA, the floating ZR-75-1 had a dose-dependent block in the S phase of the cell cycle, while the floating MCF-7 cells displayed a dose-dependent increase in apoptosis. Our working hypothesis is that BA and PBA may be interacting with integrin proteins on the cell surface of breast cancer cell lines. Integrins are heterodimeric, glycoprotein transmembrane molecules responsible for cell adhesion, survival, motility, and growth. PBA was able to show a slight inhibition of cellular attachment in growth media. In the presence of growth media containing 1 mM $MnCl_2$, integrins were stimulated to attach at a greater rate compared to non-supplemented growth media. PBA was able to statistically inhibit attachment in the presence of 1 mM $MnCl_2$.

2.P19

Mechanism of apoptosis induced by copper in PC12 cells

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Copper is an essential trace element that permits the facile transfer of electrons in a diverse manner. However, all copper compounds, unless otherwise known, should be treated as if they were toxic. Copper acts as a catalyst in the formation of reactive oxygen species and catalyzes peroxidation of membrane lipids. Moreover, recently, it has been reported that apoptosis was induced by heavy metals including copper. Apoptosis is a morphological and biochemical description of physiological cell death mechanism that is commonly associated with programmed events necessary for the differentiation and development of individuals and organs. In this study, to confirm whether copper induced apoptosis, analyses of DNA fragmentation in PC12 cells treated with copper were carried out. In addition, to determine the apoptotic pathway initiated by copper treatment, changing of apoptotic factors, such as bax, bad, and bcl-2, and caspase family in PC12 cells treated with copper were measured by Western blot and RT-PCR analyses. From the obtained results, it was shown that copper induced apoptosis in PC12 cells, and a pathway concerning copper-induced apoptosis was estimated wherein Bax, Bad and reactive oxygen

species activated the release of cytochrome *c* from the mitochondria into cytoplasm, owing to binding to apoptotic activating factor 1 and to activating caspase 9 leading to the activation of caspase 3.

2.S01

Concentrations of selected trace elements, cadmium, and steroid hormones in women with uterine myomas

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Uterine myomas make almost 95% of all benign neoplasms of the uterus. Different hypotheses suggest that myoma tumorgenesis appears to involve somatic mutations of normal myometrium and complex interactions of estrogene, progesterone, and local growth factors. Cadmium, one of the environmental factors, is recognized as a xenoestrogen. In addition, it alters the homeostasis of trace elements. The aim of our study was to assess blood concentrations of cadmium, trace elements (Zn, Cu, Mg, Ca, and Fe), estradiol, and progesterone in women with uterine myomas. The expression of ER (estrogenic) and PgR (progestagenic) receptors was also determined in tissues. Blood and tissue samples were collected from 40 women with uterine myomas, being prepared for the operation. Histopathologically unchanged tissues, collected from the same group of women, formed the control group used to assess ER and PgR expression. All the tissues were subjected to histopathological examination. Another control group composed of healthy women, confirmed by gynecological examination, was used to determine blood concentrations of metals, estradiol, and progesterone. In both the study and control groups, enhanced blood cadmium and calcium concentrations were revealed in all the women smoking cigarettes. In tissues collected from the smoking women, PgR expression was higher than in nonsmokers. In none of the groups, were there differences in blood concentrations of remaining investigated elements and hormones between smokers and nonsmokers. Only in the group of nonsmoking healthy women, the correlation between blood concentrations of estradiol and progesterone was observed. The obtained data suggests that cadmium can upregulate the internal concentration of calcium and play the role of an alternative signaling of molecule controlling various transduction pathways with mitotic or pro-apoptotic consequences. The study was supported by the Joint Project of the Medical University of Lodz (no. 502-13-483).

2.S02

Boric acid and phenyl boric acid induce apoptosis in prostate cancer cell lines

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Boric acid (BA) has been reported to inhibit the growth of prostate cancer cell lines, but the mechanism by which this occurs is unknown. In this study, three human prostate cancer cell lines: DU-145, PC-3, and LNCaP, were cultured in RPMI media in the presence or absence of 1 mM BA or 1 mM phenyl boric acid (PBA). BA completely inhibited the growth of DU-145, partially inhibited the growth of PC-3, and did not inhibit the growth of LNCaP. PBA partially inhibited the growth of all three cell lines. Both BA and PBA treatments resulted in dose-dependent cell death determined by clonogenic survival assays in DU-145 cells. Both BA and PBA caused DU-145 cells to detach and undergo apoptosis in a dose-dependent manner after 4 days of treatment. Again, both BA and PBA induced activation of caspase 3 activity, critical to apoptosis. Likewise, DU-145 cell attachment in the presence of BA or PBA was found to be inhibited. While MnCl2 was able to stimulate cell attachment, BA or PBA were independently still capable of inhibiting cell attachment in DU-145 cells. As boron is a normal component of the human diet and BA a normal constituent of plasma, foods rich in boron or a BA supplement may find application through further study to provide protection against prostate cancer.

2.S03

Zinc regulates NFkappaB and AP1 transcription factors in prostate cancer cells

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Nuclear factor kappa B (NFkappaB) and activator protein 1 (AP1) are nuclear transcription factors that regulate the expression of genes that play a role in tumor growth, angiogenesis, and cancer metastasis. Intracellular zinc appears to suppress tumorigenesis in the prostate and to induce apoptosis in prostate cell lines; thus, we investigated the effect of zinc signaling on the activation of NFkappaB and AP1 in prostate cancer cells. PC-3 cells were treated with ZnCl2 plus sodium pyrithione to increase intracellular zinc levels. Cells were collected, lysates prepared, and the levels of specific proteins analyzed by Western blot. The results showed that Zn stimulated the translocation of NFkappaB from the cytosol to the nucleus. In addition, the level of cytosolic IkappaBalpha was decreased. Zinc also increased the level of phosphorylated cJun present in the nuclear fraction. To determine whether zinc had any effect on molecules involved in metastasis, we also determined the level of MMPs in the cells. Zinc treatment decreased the expression of MMP2. All of the effects of zinc were reversed by the addition of a cell permeable Zn chelator. These results suggest that zinc signals the nuclear translocation of NFkappaB and activation of AP1. Moreover, the mechanisms likely involve zinc-induced loss of the inhibitor IkappaBalpha and phosphorylation of cJun, respectively. Supported by NIH grant CA079903.

2.S04

Effects of selenium alone and with antioxidants and ibuprofen mixture in Chernobyl catastrophe clean-up workers at risk of developing cancer

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On the night of 26 April 1986, the Soviet nuclear accident brought for the Soviet Union and Europe huge losses of financial and human resources. More than 6,500 male inhabitants of Latvia of reproductive age were involved in the clean-up and recovery work after the Chernobyl catastrophe. Consequently, they were exposed to both direct γ-radiation and inhaled or absorbed toxic radioactive isotopes and volatile heavy metals derived from the reactor melt-down through their skin, lungs, and gastrointestinal tract. After their homecoming, all clean-up workers (C-up-w) immediately visited the University Hospital for a full medical examination. The evidence obtained from studies performed to date suggest that many of the chronic diseases that are being experienced by the C-up-w, including the recently identified increase of prostate symptoms, prostate or other neoplasm, are due to the chronic manifestation of long-term oxidative stress (OS). Hence, this proposal is part of a long-term study in 134 C-up-w of age 43–55 being served. C-up-w received selenium from yeast alone or in combination with antioxidants, coenzyme Q10, ibuprofen, or placebo. Concentrations of selenium (Se), vitamin E (E), total antioxidant status (TAS), NEFA, triglycerides, lipid peroxides (LP), intensity of OS were determined. After 1 year of supplementation, results showed that increased concentrations of Se, E, and TAS, thus improved antioxidative defense of the organism and some quality of life described parameters. Simultaneously, there were decreased concentrations of LP and OS. In fact, during the supplementation period, there were decreased needs for prescribed medicines, including those for joint pain, chronic bronchitis and emphysema, stomach troubles, and depression. In groups treated for 1 year, there was no incidence of any neoplasia compared to seven new cases of neoplasia among the survey (control) C-up-w.

2.S05

Are people with low selenium and antioxidant status at much higher risk of cancer?

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A growing incidence of human cancer and other common diseases has been observed over recent decades. There is increasing evidence that numerous dietary components play a crucial role in modifying the process of carcinogenesis. Diet, the major source of vitamins, micronutrients, antioxidants, and phytochemicals, but also the source of carcinogens and mutagens, is found to be responsible for the majority of cancer deaths. Some authors proved that about 35% of cancer deaths are associated with diet, which

is comparable to the tobacco-related cancer risk. There is a growing interest in the biological role of selenium (Se) with regard to both protection of human health and prevention of diseases. Food is a major source of Se, and researchers have a 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. However, it is worth noting that evidence for Se protective role is conflicting. Epidemiological studies show that a low Se level, especially in males, is associated with an elevated risk for lung and prostate cancers. No inverse association between the Se level and cancer risk was found for lung cancer in females. In contrast to prostate cancer, breast cancer was not found to be influenced by the Se level. The majority of studies on relations between the Se level and occurrence of colorectal cancer have provided null results for both males and females. Antioxidants in general and antioxidant vitamins in particular have been studied very extensively in relation to human cancer risk. Of the 19 intervention studies, six proved the beneficial effect of antioxidants (on DNA damage), whereas in 13 studies, null effects have been reported. At present, no general conclusions can be reached on the diet-related carcinogenesis and antioxidant status (including intervention study) on cancer risk.

2.S06

Zinc and selenium status in pediatric malignant lymphomas

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Several studies on serum trace elements in malignant diseases including M. lymphomas have been reported. However, studies on hair Zinc (Zn) and Selenium (Se) concentrations are rare in pediatric M. Lymphomas (ML). The purpose of this study is to analyze Zn and Se status in ML, particularly in Hodgkin's disease (HD) and Burkitt lymphoma (BL) in childhood. Ninety-six untreated patients with ML aged from 2.5 to 16 years (81 HD and 15 BL) were studied for Zn, and 21 from this group had also selenium (Se) analysis. Their ages ranged from 2.5 to 16 years, majority of the patients were from a low SES background and were in advanced stages at admission. Plasma and hair Zn, and Se levels were measured by atomic absorption spectrophotometry before treatment. The mean plasma Zn levels in HD (75.7±5.5 μg/dl) and in BL (70.0±8.8 μg/dl) were significantly lower ($p<0.001$) compared to that of controls (109.8±12.3 μg/dl). The mean hair Zn levels (114.5±34 μg/g) in HD and in BL 93.7±23.2 μg/g were also significantly lower than the controls (184.0± 19.3 μg/g). Plasma and hair Se levels were 42±7 ng/ml and 290±37 ng/g in HD and 36±4 ng/ml and 233± 13 ng/g in BL, respectively, both significantly lower than that of controls ($p<0.001$ and $p<0.01$). In conclusion, chronic Zn and Se deficiencies (low plasma and low hair Zn and Se levels together) were found to be associated with ML patients in Turkish children before treatment. The majority of the patients were from low SES which may be partially responsible from low Zn and Se status. Supplementation of the patients with these trace elements in addition to standard chemo-radiotherapy regimens was suggested.

2.S07

Anticancer activity of selenium-enriched broccoli sprouts in human prostate cancer cell lines

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Anticancer effect of selenium-enriched broccoli sprouts was investigated in the human prostate cancer cell lines. The selenium-enriched broccoli sprouts were germinated in sodium selenite solution, and the speciation of selenium compounds was performed using high performance liquid chromatography (HPLC)/inductively coupled plasma-mass spectrome-

try (ICP-MS) with size exclusion column. Analysis of sulforaphane, the natural anticancer agent in broccoli sprout was performed using HPLC with reversed phase column. Selenium-enriched broccoli sprout extract was then applied on human prostate cancer cell line (LNCaP, PC-3 and DU-145). Cell proliferation inhibition was observed using MTT assay, while prostate-specific antigen (PSA), a well-accepted prognostic indicator of prostate disease, was analyzed immunoenzymatically. The most abundant form of selenium in selenium-enriched sprouts was Se-methylselenocysteine. There was no differential sensitivity of both selenium-enriched and normal broccoli sprouts in prostate cancer cells at higher concentration. On the other hand, in the lower concentration, the effect of selenium-enriched broccoli sprouts in inhibiting cell proliferation and PSA secretion was superior then normal broccoli sprouts. We speculate that the same effect given by both sprouts in higher concentration was due to sulforaphane effect, as an active compound in the normal broccoli sprouts, while in the lower concentration, the anticancer effect of sulforaphane was increased by the presence of selenium compounds.

2.S08

Adding iron to white tea may decrease its antioxidant capacity in humans

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We have proposed the hypothesis that iron may interact with polyphenols in the lumen and, thereby, modulate their antioxidant ability in vivo. Lumenal interactions of polyphenols with iron are frequently addressed in studies of iron absorption. Presumably iron–polyphenol chelates are formed during digestion, and this results in dramatically lower iron absorption. It follows that formation of iron–polyphenol chelates may inhibit the absorption of polyphenols. We tested in humans the hypothesis that adding iron to tea diminishes the increase in antioxidant capacity in plasma, expected from tea ingestion. Ten women, aged 25–55 years, received white tea infusion or a mixture of white tea infusion and iron lactate in a randomized cross-over design. Blood samples were drawn before the administration of and 30, 45, and 90 min after the consumption of the test drinks. The antioxidant capacity of plasma was measured with the ferric-reducing ability of plasma (FRAP) assay. The phenolic content of plasma was measured as total catechins and as content in selected phenolics with an HPLC assay. White tea increased the antioxidant capacity and the phenolic content of plasma ($p<0.0001$) in a time-dependent manner ($p<0.0001$). Iron inhibited the rise in antioxidant capacity and in the phenolic content of plasma ($p=0.0057$). These results suggest that iron may modify the antioxidant properties of polyphenols and are in support of the hypothesis. Potential inhibition of polyphenol absorption as a result of lumenal interactions between iron and phenolics is a plausible mechanism that may explain the observed effect and reserves further investigation. This study elucidates the complex lumenal interactions between iron and polyphenols. The evaluation of the overall antioxidant capacity of the complex food systems contributes to the discussion on the optimal intakes of polyphenol and iron.

Category 3: Cardiovascular and pulmonary diseases

3.P01

Blood antioxidant markers and elemental levels in Azorean patients with coronary artery disease: a preliminary study

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Atherosclerosis (ATH) and related vascular diseases are the first cause of morbidity and mortality in Portugal, including the Azores' Archipelago. The oxidation of low density lipoproteins (LDL) is considered to be an important event in the development of the disease. Accordingly, by preventing LDL oxidation, antioxidants can possibly reduce the risk or the progression of

ATH. Essential trace elements as Cu, Se, Zn, and Fe are involved in endogenous antioxidant systems, mainly as cofactors of antioxidant enzymes. Other elements, like K and Ca, might play a role in atherogenesis while implicated in physiological and metabolic processes which are known to be disturbed in ATH. The aim of the present study was to evaluate some blood antioxidant markers and blood elemental levels in 20 Azorean subjects (ten men and ten women, aged 40 to 65 years) with coronary artery disease previously submitted to percutaneous revascularization (PCI). The whole blood gluthatione peroxidase (Se-GPx) and erythrocyte superoxide dismutase activities were measured, as well as serum vitamin E levels (by HPLC). The concentrations of K, Ca, Fe, Cu, Zn, and Se in plasma and blood cells were determined by PIXE. Results were analyzed by taking into account several cardiovascular risk factors, namely, gender, hypertension, dyslipidemia, and total plasma homocysteine (tHcy) concentration. Se-GPx activity and vitamin E levels were significantly lower (24 and 15%, respectively) in the PCI group than in controls. In blood cells, significantly differences in Ca (19±5 vs 24±6 μg/mL) and Zn (14±1 vs 12±2 μg/mL) levels were observed in PCI women (but not in men) compared to the respective counterparts. Also in PCI women, both serum triglyceride and plasma tHcy concentrations were above reference values and higher than in men. Results suggest a decreased antioxidant status in PCI patients. In particular, women appear to be less protected than men, as reflected in some cardiovascular risk factors.

3.P02

Blood trace element levels in patients with stabilized atherosclerosis

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Cardiovascular diseases remain the leading cause of mortality in Western populations. Dislipidemia, hypertension, diabetes, obesity, and tobacco smoking are the main risk factors for atherosclerosis and its thrombotic complications. However, these factors alone cannot account for all of the deaths caused by vascular pathologies. The possible interaction of some trace element levels with atherogenic diseases is not completely clarified. On the other hand, the intra- and extracellular concentrations of elements playing an important function in the electrolytic equilibrium could partially be associated with cardiovascular dysfunctions and other associated pathologies. The aim of this study was to evaluate trace elements (Fe, Ni, Cu, Zn, Se) and electrolytic-balance-associated elements (K and Ca) in plasma and blood cells of patients with stabilized atherosclerosis. Significant differences between patient and control groups were observed in blood cell K, Fe, and Zn concentrations, while plasma K, Ca, Fe, and Zn levels exhibited significant differences between groups. Results will be discussed taking into account the main function of those elements and the etiology of atherosclerosis. The relationship of results with the studied cardiovascular risk factors will be also considered.

3.P03

Magnesium in the serum of patients treated surgically

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Magnesium is one of the most important elements, fulfilling significant and complex functions in the body. Changes in concentration of magnesium in the serum can be linked with the occurrence of many varied pathological states. The aim of this work was to present some findings from our studies that we carried out earlier concerning concentration of magnesium in serum in patients with hypertension, disorders of the synostosis, thrombophlebitic complications of the lower limbs, after removal of the gall bladder by laparoscopy or laparotomy, with disturbances of the rhythm of the heart. In all the mentioned groups of patients with postoperative complications,

lowering of magnesium concentration in relation to normal values was ascertained. For example, average concentration of magnesium in patients (n=59) with hypertension (of varying degree of severity) was 15.1, and in the control group (healthy persons), 20.3 mg/L, with disorders of the synostosis (n=29) 15.5, in the control group (patients without synostosis disorders)—19.2 mg/L. In patients with thromboembolic complications after cholecystectomy (n=89), average concentrations of magnesium were 14.3, while in patients in whom thrombosis was not present, 18.7 mg/L. The magnesium concentration was measured by flame atomic absorption spectrometry. The accuracy of the method was verified against a serum reference standard SeronormTM Trace Elements SERO AS Billingstad, Norway. The presented paper and results can contribute to the data on magnesium concentrations in serum of patients with different complications after surgical intervention.

3.P04

Zinc deficiency decreases erythropoiesis in rats: implication for possible zinc-deficiency anemia in humans

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Iron medication, a major cure for anemia, is not effective for some subsets of nutritional anemia, i.e., iron refractory anemia. Some researchers reported that oral zinc effectively cured some patients with iron refractory anemia. It is not established that single zinc deficiency induces anemia in humans. We propose a hypothesis that zinc deficiency impairs production of erythroid cells. To test the hypothesis, 30 3-week-old male Sprague–Dawley rats were assigned into three groups of ten for the 4-week study receiving AIN-93G-based EDTA-treated casein diet: ad libitum fed control group (AIN-93G), moderate zinc deficiency group (4.5 mg Zn/kg diet), and pair-fed group (AIN-93G). Spleen weights, plasma erythropoietin concentration (EPO), complete blood counts, reticulocyte counts (Ret), and red cell distribution width (RDW) were measured, and peripheral blood and bone marrow smears (Wright-Giemsa stain) were observed. Zinc deficiency per se induced hypoplastic bone marrow and anisocytosis and spherocytosis in peripheral blood; significantly diminished Ret and increased relative spleen weight. Zinc deficiency per se also significantly increased RDW and decreased plasma alkaline phosphatase activity. Diet restriction significantly increased MCV and decreased plasma EPO. Changes in MCV and plasma EPO were similar in moderate zinc deficiency group and in pair-fed group. These results suggest that prolonged moderate zinc deficiency may cause anemia due to impaired erythropoiesis. Therefore, individuals with zinc deficiency anemia might be included in patients with iron refractory anemia. This research was financially supported by Japan Science Society (The Sasakawa Scientific Research Grant no. 18-144).

3.P05

High-dose zinc to terminate angina pectoris: a review and hypothesis for action by ICAM inhibition

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Lipid peroxidation and LDL oxidation are believed to be critical for arteriosclerosis and, consequently, angina pectoris and Raynaud's disease. Administration of biologically available zinc (zinc gluconate) was a beneficial treatment for angina pectoris and Raynaud's disease in several patients during a 1983 zinc lozenge for common cold clinical trial at doses varying from 150 to 300 mg per day. Follow-up showed that they remained asymptomatic. Other patients were similarly treated from 1983 to the current date in a clinical setting with similar results. Zinc blocks calcium and its several actions on atherogenesis. Increased amounts of cytotoxic cytokines such as TNF-alpha, IL-beta and IL-8, often produced in the elderly, are blocked by high-dose zinc in humans. We hypothesize that higher serum concentrations of LDL cholesterol resulting from administration of 300 mg of zinc per day as observed by Chandra is caused by a release of low-density cholesterol from cardiovascular tissues, beneficially flushing it into the serum where it is readily observed, thus decreasing arteriosclerosis, increasing circulation, terminating angina pectoris and restoring more youthful cardiac function, and that such is not

harmful. Although prevention of cholesterol-induced arteriosclerosis by zinc is predicted from findings related to oxidative stress and lipid peroxidation, removal of LDL might be attributable to action of ionic zinc on ICAM inhibition. In stark contrast to current practice, high-dose zinc should be considered as basic in the strategy of prophylaxis and therapy of the atherosclerosis process to terminate angina pectoris and restore youthful cardiac function.

3.P06

Lipidemic profile and heavy metal body burden in hypertensive patients

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Lipidemic profile is strongly correlated to cardiovascular pathology and arterial hypertension. Essential trace metals like zinc and copper are also strongly correlated to collagen and elastin biosynthesis of cardiovascular system and hence to hypertension and coronary disease. Dietary habits are the main sources of the above metals in human body. The aim of the present study is to investigate correlations between lipidemic profile and heavy metal body burden of hypertensive patients living in the same urban area with common nutritional and lifestyle habits. Seventeen (11 male, 6 female) hypertensive patients, aged 35–55 years under monotherapy or combination of antihypertensive treatment [b-blockers, angiotensin-converting enzyme inhibitors (ACEI), calcium inhibitors (CaI)] were analyzed for blood serum Chol, HDL, LDL, Tg, Na, K, Ca, P, Zn, Cu, Cd levels and hair Zn, Cu, Cd content. Systolic and diastolic blood pressure before and after antihypertensive treatment, along with their nutritional history, were registered. Our results revealed better lipidemic profile for treated women patients compared to men. Mean serum Zn/Cu ratio was found increased in combined ACEI/CaI-treated patients. Hair zinc/copper ratio, although indicative of the chronic metal body status, was found significantly higher compared to normal values in both sexes (Zn/Cu:men:25,53, women:15,58, NL:4-12. Serum Cd levels were nearly 40% less in women compared to men. Hair Cd concentration was within normal limits in both sexes, but in women, a positive correlation may be observed to HDL/ LDL ratio. In conclusion, nutritional- and lifestyle-originated metals may play a significant role in hypertensive disease onset and treatment (Supported By the EC E.P.E.A.E.K. II “HRAKLEITOS” (61/1783) Project).

3.S01

Fish intake, fatty acids, mercury, selenium, and risk of myocardial infarction—design of a prospective case-control study

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A majority of previous studies on fish intake and myocardial infarction indicate a protective effect of fish intake; probably an effect of the long-chain fatty acids in fish. But not all studies indicate a protective effect of fish intake. There are studies showing a connection between high levels of mercury, a contaminant in some fish, and an increased risk of myocardial infarction. The aim of the study is to further elucidate whether intake of fish, mercury, selenium or the sum of proportions of fatty acids EPA (20:5$n-3$) and DHA (22:6$n-3$), influence the risk of myocardial infarction. Within a population-based cohort from a community intervention programme, 503 cases of myocardial infarction and 626 matched controls were identified and included in this nested case-control study. Information on fish intake had been recorded at recruitment, i.e., before diagnosis. Mercury and selenium levels will be determined in erythrocyte membranes, and the relative content of fatty acids will be measured in plasma phospholipids, also collected at recruitment. Previous studies of the same population have shown intercorrelations between the biomarkers associated to fish consumption.

Therefore, a multivariate approach will be applied. By October, we will have preliminary results at hand.

3.S02

Effect of selenium supplementation on heart lesions produced by neuroleptics

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Neuroleptics can induce organic or functional heart lesions that may lead to sudden patient death. As selenium (Se) has previously been suggested to play a role in the development of cardiomyopathy, the present study was undertaken to determine whether neuroleptic-induced lesions correlate with low heart Se and whether Se supplementation has an effect on cardiac lesions. Forty NZW adult rabbits were separated into four groups: the control group (GI) received a saline solution, GII was treated with levomepromazine (3 mg/kg/day) and risperidone (1 mg/kg every other week), GIII received sodium selenite and GIV received levopromazine + risperidone + sodium selenite (GIV). All treatments were administered by intramuscular injection. At the end of the 3-month treatment period, myocardial Se was measured in all animals, and their hearts were examined histologically. Myocardial Se levels in treated animals (GII; $p<0.001$) were half those in controls (GI). In contrast to control animals (GI), treated animals (GII) developed heart lesions including disorganization of cardiac fibers, myolysis, interstitial and endocardial fibrosis, and necrosis. After 90 days of Se supplementation in neuroleptic-treated rabbits (GIV), heart Se was less significantly decreased than in GII with a reduction of necrosis and fibrosis. However, Se-supplemented rabbits (GIII) showed heart lesions differing from those observed in GII, such as nuclear dystrophy, reflecting the toxicity of Se in organs with a sufficient supply. In this study, Se supplement reduced the number and severity of heart lesions showing it to be beneficial and associated with protection of the heart in neuroleptic-treated rabbits. Our results support the hypothesis of a correlation between neuroleptic-induced heart lesions and decreased myocardial Se levels.

3.S03

Micronutrient treatment improves cardiac damage in mice infected with *Trypanosoma cruzi*

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Effects of free radicals as causal agents in cardiac lesions have been showed in the last few years. Enzymatic and non-enzymatic antioxidants protect living organisms against the attack of reactive oxygen species. Minerals which exert antioxidative function include selenium (Se), zinc, copper, and manganese. It has been reported that the correlation between low serum Se levels and the incidence or severity of heart diseases and positive results have been found with its supplementation such as: coronary heart disease and myocardial infarction in subjects from Finland; cardiomyopathy in patients nourished by parenteral via congestive heart failure in children malnourished; decreasing in left ventricular end-diastolic volume in hamster heart. In addition, our group investigated the effect of Se supplementation in mice infected with *Trypanosoma cruzi*, after the knowledge that Se deficiency decreased the resistance to experimental infection, and that low Se levels were associated with the severity of cardiopathy in chagasic patients. We verified that Se prevented the heart from inflammatory damage driven by *T. cruzi* infection in mice in different phases of the infections as shown by biochemical and echocardiograph data. Future histological studies are important to confirm the potential benefit of this micronutrient treatment on chagasic cardiopathy in mice.

Category 4: Diabetes mellitus, metabolic syndrome X, and obesity

4.P01

The role of low doses of lead on development of dislipidemy: experimental data

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The link between environmental pollution and premature ageing, the decrease of life expectancy and higher morbidity levels among people living in harsh environmental conditions is now well established. Dislipidemy remains the risk factor for atherosclerosis and cardiovascular diseases. Even though clinically manifested forms of lead intoxication are now rarely detected, the problem of lead exposure in low doses remains important taking into account the peculiarities of lead accumulation and toxicodynamics. Lifetime lead accumulation in depot and ability of lead to be released from depot in certain conditions, e.g., as a result of osteoporosis in postmenopausal period, can accelerate formation of metabolic syndrome. The aim of the current study was to establish the possibility of development of dislipidemy under lead exposure in low doses. Young (3–3.5 months) and old (22–23 months) Wistar rats were intraperitoneally injected with 1.53 mg/kg lead acetate. After 50 injections, lead levels in blood were measured using atomic absorption spectrometry; Zn-protoporphyrine, total lipids, cholesterol, triglycerides, phospholipids and β-lipoproteides were measured by standard methods. After 50 injections of lead acetate, the lead content in blood increased 4.5 times in young rats and 1.8 times in old rats compared to the control groups. Zn-protoporphyrine increased 3 and 2.5 times, respectively. These data indicate disturbances of heme synthesis and iron utilization. Dislipidemy in old rats was characterized by increased levels of cholesterol to 62.6% and β-lipoproteides to 52.9% compared to the control. These indices remained almost unchanged as well as triglycerides content in young rats. The increase of phospholipids was detected in both groups two times. Lead accumulation in rats after exposure to low doses during 50 days caused disturbances of heme synthesis and development in dislipidemy among old rats.

4.P02

Is plasma chromium concentration a predictor for favourable effect of chromium supplementation?

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The presence of chromium (Cr) in man has been shown to be necessary for maintaining normal carbohydrate and lipid metabolism, and chromium supplementation could be beneficial for the people who do not have enough Cr in the body. There is still no good parameter which would indicate Cr insufficiency. The aim of our study was to investigate the effects of Cr suplementation on parameters of carbohydrate and lipid metabolism in patients with type 2 diabetes and to find any marker which could predict a favorable effect of Cr supplementation on insulin resistance and HbA1c. In the randomized, double-blind, placebo-controlled cross-over trial, we included 60 patients with type 2 diabetes treated only by diet. They received 3 months chromium picolinate 1,000 μg daily and 3 months placebo. We observed: HbA1c, fasting and postprandial plasma glucose concentration, fasting and postprandial insulin concentration, body weight, HOMA-IR, blood concentrations of total, HDL and LDL cholesterol, triglycerides and Lp(a), the chromium concentrations in urine, plasma and erythrocytes. The chromium concentration in urine, plasma and erythrocytes was measured with ETAAS with Zeeman corrections. According to the poor effect of chromium on the parameters of carbohydrate and lipid metabolism, we found 35 patients with lowered HOMA-IR and 15 with lowered HbA1c after CrPic supplementation. With logistic regression, we did not find any indicator for patients in whom we could expect the lowering of HOMA-IR (p=0,593), but we found the elevated chromium concentration in plasma as predictor for good effect of CrPic supplementation

to HbA1c (p=0,039). Among the measured concentrations of chromium in the urine, plasma and erythrocytes, the elevated plasma chromium concentration was found as a marker which could show us potential responders to whom CrPic supplementation could result in lowering of HbA1c.

4.P03

Longitudinal hair-Zn profiles of elderly subjects with normal glucose tolerance and type II diabetes mellitus

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Evidence exists for the role of zinc in glucose metabolism, although the mechanism of action is not fully understood. The aim of this study was to investigate the effects of glucose intolerance on Zn metabolism. Hair Zn profiles were measured in a group of healthy elderly subjects (N=48, M=30, F=18) and NIDDM patients (N=58, M=35, F=23). Zn content of the proximal 3 mm of 30–50 hairs (H-Znpr) and the average Zn content of the 25 mm hair length (H-Znav) were obtained from the data. Variation of Zn content along the whole hair length was also calculated. The latter was assumed to be representative of Zn metabolized in the body. Some evidence for this is presented. No statistically significant difference was found between the two groups in H-Znav. The frequency distribution of log H-Znpr, data of both groups, showed a definite bimodal pattern. Approximately 16% of subjects had low H-Znpr content (median 50 μg/g), while the majority (84%) showed normal H-Znpr. (median 141 μg/g) irrespective of the population group examined. In comparison to the controls, NIDDM patients displayed a narrower distribution of H-Zn values. Unlike Cr, a relatively small variation (14–15%) in Zn content along the hair length (2–4 months in time scale) was observed, and no statistically significant difference existed between the two groups. A significant positive association (r=0.3847, N=40, p<0.05) between duration of illness and fasting plasma glucose was previously found. A similar weak (r=0.3195, N=23, p>0.05) positive correlation was established between duration of illness and H-Znav. Neither of these two population groups exhibited an association between H-Znav and age. The results presented suggest that in contrast to Cr, Zn metabolism is not influenced by glucose oscillations characteristic of NIDDM patients. However, an increased Zn metabolic rate was observed as the illness progressed. This implies that increased fasting plasma glucose requires more Zn to be metabolized.

4.P04

Involvement of macro- and trace elements in dysregulatory mechanisms of obesity and type II diabetes pathogenesis in pre- and postmenopausal women

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Recently, we (Skalnaya, Demidov, Skalny, 2006, 2007) found BMI to be significantly correlated with macro and trace element hair contents in men and women. Mainly, overweight and especially obese subjects have decreased hair content of Mg, Zn and increased K, Na, Pb, Al. In this study, the scalp hair mineral contents of obese (group O, n=141) and diabetic (type II; group D, n=93) pre- and postmenopausal women, analyzed by ICP-OES/ICP-MS, were compared to the control group (group C, n=1236). The elevated hair Hg (p<0.02), K (p<0.05) and lower hair Ca, Mg, Zn, I in group O were found. Group D differs from control by decreased Ca, Mg, Zn, Co, I and increased hair K, Na, Hg. So, hair mineral profiles in obese and diabetic women were very similar except Na content (elevated in group D). But the severity of deviations in obtained data was more significant in diabetic women compared to obese ones. The pathophysiological mechanisms of obesity and type II diabetes in pre- and postmenopausal women are similar, and using of macro and trace elements in prevention and treatment of both pathologies is essential.

4.P05

Determination of heavy metals and trace metals in ground water and soil in Libya by flame AAS and heart and diabetics disease

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Libya depends on ground water, rain water, and a desalinization program (to be started). Our research was to assay the levels of copper, lead, cadmium, and other metals in ground water and in soil. Correlation to these metals was investigated by using different sites and locations. Different extraction methods were used, and EDTA proved to be a good extractant of metals from soil. Different methods were used and discussed. We found a correlation between zinc, calcium, and magnesium, and disease. We compared different cities in Libya to find correlations for such diseases. Also, we find that a large number of diabetics related to zinc and chromium deficiency. Statistical analyses are also given for this study. This is an original study, and we compared results from different countries. The hardness of water is considered important for heart disease. Therefore, we concentrated on these factors in our study.

4.P06

Ages and copper ions

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We investigated the propagation of glycation process in the presence and absence of 0.1 mM Cu^{+2} on native collagen by in vitro incubation with glycated BSA, for 10 days and 6 weeks. The glycated BSA was obtained by incubation of 100 mg/ml BSA with 1.6 M glucose. The SDS-PAGE and gel filtration analyses showed the presence of 85.9 kDa AGE-BSA monomer and a 162.6-kDa dimer, whereas the unglycated monomer of BSA was 67.9 kDa. The elution pattern of control sample (non-incubated mixture AGE-BSA and collagen) in the presence or absence of Cu^{+2} showed three important peaks corresponding to the collagen peptides (34.6 kDa), the AGE-BSA monomer and dimer. After 10 days of mixture incubation in the absence of Cu^{+2}, the collagen peptides peak, and the AGE-BSA dimer peak showed a slight increase in molecular weight and a twofold increase in UV absorbance and specific fluorescence compared to control. In the presence of Cu^{+2}, the elution pattern showed the appearance of a new 21.5 kDa peak with high fluorescence, the decrease of the molecular weight of collagen peptides until 30.6 kDa, and the disappearance of AGE-BSA dimer. After 6 weeks of glycation in the absence of Cu^{+2}, a lot of cross-linking compounds with molecular weight between 59.6 and 401.46 kDa with a maximum level of fluorescence and UV absorbance associated to the 110 kDa product appeared. The presence of Cu^{+2} generated a molecular aggregate with molecular weight between 34.1 and 375 kDa with a maximum of fluorescence and UV absorbance for the 34.1 kDa product. Our experiments have shown the progression of cross-linking of unglycated proteins in the presence of glycated proteins but in the absence of sugar. The ions induced initially a protein fragmentation probably due to ROS generation. The progression of glycation was possible by the degradation of existing early and advanced glycation products in the presence of Cu^{+2} that generated ROS and other more reactive products that can attack the unglycated proteins.

4.S01

Female offspring of rat dams fed low-boron diets during pregnancy and lactation exhibit signs of the metabolic syndrome during early adulthood: increased body weight, and serum triglycerides and total cholesterol concentrations

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To expand on findings from this laboratory that low dietary boron may affect energy substrate utilization, we

determined whether low dietary boron during early development promotes manifestation of the metabolic syndrome in adult offspring. Sprague–Dawley dams were fed either a boron-low (BL; ~0.1 mg B/kg) or boron-supplemented (BS; ~3.0 mg B/kg) diet from 75 d before breeding (with diet-matched males) to weaning of pups. Female weanlings (21 days of age; 24/group) were re-randomized and fed either the BL or BS diet for 63 days. Beginning at 28 days postweaning, maternal boron deprivation, but not postweaning boron nutriture, increased body weight (p=0.0001) in offspring (215 vs 192 g at 84 days of age). Also, at 84 days of age, maternal boron deprivation increased serum concentrations of triglycerides (0.54 vs 0.47 mmol/L, p= 0.005) and total cholesterol (2.04 vs 1.89 mmol/L, p= 0.004) and decreased alkaline phosphatase (121 vs 146 U/L; p=0.004). Independent of maternal nutriture, postweaning boron deprivation increased serum concentrations of glucose (11.2 vs 10.1 mmol/L; p= 0.001). The findings that a maternal diet limited in boron content (i.e., one low in fruit, nut, vegetable, and legume content) may increase manifestation of some components of the metabolic syndrome in adult offspring, including an 11% increase in body weight, suggests a possible role for boron in prevention of the syndrome.

4.S02

Safety, absorption, and antioxidant effects of chromium histidine

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Supplemental chromium has been shown to be involved in the alleviation of the metabolic syndrome, glucose intolerance, polycystic ovary syndrome, depression, excess body fat, and gestational, steroid-induced, and type 2 diabetes. Chromium amino acid complexes that contained histidine displayed consistently higher absorption compared with other chromium complexes tested in human subjects, and a complex of chromium tri-histidine displayed the highest absorption. Previous studies have shown, using purified DNA, that chromium histidine displayed significantly lower DNA damage (similar to control cells) than other commercially available chromium nutritional supplements. We used human cultured HaCaT keratinocytes and also were unable to find any signs of toxicity of the chromium histidine complex. The trivalent forms of chromium tested exhibited protective antioxidant effects when cells were exposed to hydrogen peroxide-induced stress. In chromium-histidine-treated cells, transcripts related to the antioxidant family were upregulated. When HaCaT keratinocytes were preincubated with chromium histidine before peroxide, there was increased expression of genes implicated in oxidative repair (POLD2) and antioxidant defense (GSS), whereas the SOD2 transcript was downregulated. These results demonstrate that chromium histidine is a safe form of chromium that is absorbed by humans better than any of the known chromium complexes and functions as an antioxidant. Chromium histidine is part of U.S. Patent no.6,689,383 "Chromium–Histidine Complexes As Nutritional Supplements", USDA License no. 1367-002.

4.S03

Zinc and iron absorption and nutritional status after gastric bypass in morbid obese patients

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The objective of the study was to evaluate zinc and iron absorption and nutritional status in patients with morbid obesity before and after 6 and 12 months of gastric bypass (GBP). Sixty-seven women were studied previous to gastric bypass (average age 36.7± 9.8 year, weight 115.1±15.5 kg, BMI 45.2±4.6 kg/m^2). In 47 and 28 individuals, there were results after 6 and 12 months of surgery, respectively. Zinc status was assessed through the determination of plasma

zinc (PLZn), plasma alkaline phosphatase activity (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 was evaluated in a subsample of 15 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. A significant reduction of zinc absorption from a standard diet was observed from 34.7% before the surgery to 18.8% after 6 months, and to 18.5% after 12 months of GBP ($p<0.02$). Iron absorption decreased from an initial value of 10.5 to 3.0% after 6 months and to 2.6% after 12 months of GBP ($p<0.001$). Indices of Zn and Fe before surgery were (mean±SD): PLZn 87.2±11.1 μg/dL, AP 36.7±7.5 U/L, EZP 263.6± 33.6 mg, Hb 13.7±0.9 g/dL, FEP 61.1±19.6 μg/dL RBC, SF (geometric mean) 37.0 μg/L. After 12 months of GBP, Hb, SF, and EZP were markedly decreased ($p<0.05$). No major changes were observed regarding PLZn, AP, and FEP. Gastric bypass in morbid obese patients was associated with decreased intestinal absorption of iron and zinc. Iron and zinc nutritional status were significantly impaired in the patients after this type of surgery (Funded by FONDECYT project 1040765).

Category 5: Disorders of aging

5.P01

Elements in hair of population with different grade of hypothyroidism

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Elemental hair analysis provides early information on relations, deficiency or excess of elements, and improve the understanding of pathochemical pathways of disadaptation, beginning, and development of different diseases. As diet and environmental stress plays a major role in thyroid health, here, assessment was extremely useful when multiple causes act in synergy for diagnostic and treatment strategy. In this study, we investigated 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 of 72 adult subjects with different ranges of hypothyroidism. For diagnostic functional status of thyroid gland and other organs of interest we used bio/resonance diagnostique and the status of thyroid hormones T3, T4 and TSH. On the basis of obtained results, most of subjects suffering from low-grade hypothyroidism, undetectable by blood tests because thyroid hormones are inside normal range. Hair analysis data of all subjects with low grade hypothyroidism showed that they suffer from deficiency of: I, Co, Zn and in lower extent, Se, and have excesses of Ca and Mg, and in lower extent, Si. The next group represents subjects with mild developed hypothyroidism, with border values for thyroid hormones. They, besides I, Co, Zn, and Se deficiency suffer from Mn deficiency and have, besides Ca, Mg, and Si, in excesses Ni, Cu, and K. The last group represents subjects with detectable disturbances in thyroid hormone function and with highest extent of disturbances of large number of elements in hair.

5.P02

The consequences of aluminium exposure on rat genital organs and sexual accessory gland histoarchitecture: three generation experiment

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The aim of the study was to evaluate the consequences of chronic aluminium intake on genital organs and sexual accessory gland histoarchitecture. The study was carried out on 28 white Wistar male rats divided in three experimental groups (E) and one control (C) group. The experiment was realized in three steps. (1) The individuals from E groups received aluminium as aluminium sulphate in drinking water as follows: E1: 200 ppb Al; E2: 400 ppb Al, E3: 1,000 ppb Al (levels determined in drinking water for animals in aluminium industry surrounding areas), C: tape water (~50 ppb aluminium). (2) Two males from each E groups were mated with female

unexposed to aluminium (ratio: one male is to two female) to obtain F1 generation. During pregnancy, the females were exposed to the same aluminium levels; the males from F1 generation were exposed to aluminium in utero, during lactation until weaning and then, 3 months to 200 ppb (E'1), 400 ppb (E'2) and, respectively, 1,000 ppb (E'3) Al. 3. The protocol for F2 generation obtained was similar to those of F1 generation. After 3 months of exposure, males from each group and generation were euthanatized, and studied organs samples were collected and, for histological examination, stained by Mallory method. Exposure to aluminium determined in testis: interstitial edema, basal membrane disintegration, seminiferous tubule necrosis more evident in F2 generation; in epididymis: interstitial edema, epithelial necrosis and exfoliation; in seminal vesicles: partial necrosis and epithelial exfoliation; in prostate: prostatic epithelial necrosis and in individuals exposed to 1,000 ppb presence of amyloidal mass; in bulbo-urethral glands: epithelial necrosis and exfoliation. The histoarchitecture of genital organs and sexual accessory glands were damaged, which is more evident in F1 and F2 generations.

5.P03

Age-related antioxidant defense weakening is altered by low selenium intakes

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Selenium is a trace element playing an important role in the machinery that protects cell components from oxidative damage. Numerous studies that deal with long and/or high selenium depletion, report deleterious effects at the cellular level, associated with an increase of pathology occurrence. The aim of the present study was to determine whether 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. Some selenoprotein activities are highly inhibited (GPx, MsrB1), and level of oxidized protein increased in both skeletal muscle and heart. These changes are exacerbated during aging. Using a 2D electrophoretic method, changes of protein expression patterns have been also 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 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 concerning aging, can be raised. Further research, clinical experiments, epidemiological studies, could help and contribute to a high accuracy of estimations.

5.P04

The consequences of female rat exposure to lead acetate on some sexual hormones

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The aim of the study was the evaluation of lead reproductive toxicity for female rats. The study was carried out on 28 white Wistar female rats divided in four groups, three experimental (E) and one control (C) exposed to lead acetate in drinking water, 3 months, as follows: E1: 50 ppb, E2: 100 ppb, E3: 150 ppb and C: tap water (0 ppb). Biological markers, the sexual hormones: FSH, LH, estradiole, progesterone, and testosterone that emphasize the disrupting potential of reproductive function were evaluated at the end of experiment, by ELISA technique. The estimation was carried out in all groups in proestrus, where there is a hormonal peak for majority of studied sexual hormones and at the some hour (around 1 P.M.). The consequences of exposure to lead acetate were: no influence on seric FSH level dynamics; significant increase of seric LH level comparative to C group and physiological limits and not conclusive variation related to exposure level; significant increase of seric estradiole level comparative to C group and physiological limits but significant decrease at treble

exposure level remaining over physiological limit; significant increase of seric progesterone level comparative to C group but not over in physiological limits and significantly indirectly correlated to exposure level, significant increase of seric testosterone level comparative to C and not correlated to exposure level dynamics.

5.P05

The consequences of female rat exposure to aluminum sulphate on some sexual hormones

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The aim of the study was the evaluation of aluminum reproductive toxicity for female rats because of poor information in the field. The study was carried out on 28 white Wistar female rats divided in four groups, three experimental (E) and one control (C) exposed to aluminum as aluminum sulfate in drinking water, 3 months, as follows: E1: 200 ppb, E2: 400 ppb, E3: 1000 ppb Al and C: tape water (~50 ppb Al). Biological markers, the sexual hormones: FSH, LH, estradiol, progesterone, and testosterone that emphasize the disrupting potential of reproductive function were evaluated at the end of experiment, by ELISA technique. The estimation was carried out in all groups in proestrus, where there is a hormonal peak for majority of studied sexual hormones and at the some hour (around 1 P.M.). The consequences of exposure to aluminum were: no influence on seric FSH level dynamics; significant decrease ($p<0.05$) of seric LH level comparative to C group, not significant and not correlated to exposure level dynamics but physiological limits (1); significant ($p<0.05$) increase of seric estradiol level comparative to C group (exception in E1 group where was lower) and physiological limits, direct correlated to exposure level, higher than physiological limit; significant ($p<0.01$) increase of seric progesterone level comparative to C group, decrease inversely correlated to exposure level but in physiological limits; significant ($p<0.01$) increase of seric testosterone level comparative to C group and indirect decrease but not significant to exposure level.

5.P06

The consequences of lead exposure on rat genital organs and sexual accessory glands histoarchitecture: three-generation experiment

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The aim of the study was to evaluate the consequences of chronic lead intake on genital organs and sexual accessory gland histoarchitecture. The study was carried out on 28 white Wistar male rats divided in three experimental groups (E) and one control (C) group. The experiment was realized in three steps. (1) The individuals from E groups received lead as lead acetate in food briquettes as follows: E1: 1,000 ppm; E2: 2,000 ppm, E3: 3,000 ppm (levels representing LOAEL—Ayar and al. 1973 quoted by Descartes, 1986, double and triple LOAEL for reproductive function), C: no lead supplement. (2) Two males from each E groups were mated with female unexposed to lead (ratio: one male is to two females) to obtain F1 generation. During pregnancy, the females were exposed to the same lead levels; the males from F1 generation were exposed to lead in utero, during lactation until weaning and, then, 3 months to 1,000 ppm (E'1), 2,000 ppm (E'2) and, respectively, 3,000 ppm (E'3) lead acetate. (3) The protocol for F2 generation obtaining was similar to those of F1 generation. After 3 months of exposure, males from each group and generation were euthanatized, and studied organs samples were collected and Thricromic Mallory stained. Exposure of male rats to lead during three generations determined: in testis: interstitial edema, seminiferous tubules epithelial necrosis, and exfoliation, Leydig cell necrosis, seminiferous tubules necrosis; in epididymis: epithelial necrosis and exfoliation, basal membrane segmental necrosis; in prostate: interstitial edema, epithelial necrosis and exfoliation, presence of amyloidal mass in individual exposed to 3,000 ppm lead acetate; in seminal vesicles: partial necrosis and epithelial exfoliation; in bulbo-urethral glands: no important lesions were observed. The results are similar to those of Murthy et al. (1991) and Boscolo et al. 1988, quoted by Apostoli et al. (1998).

5.S01

Serum selenium, copper, and zinc in elderly population from coastal and continental areas of Croatia

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In 1969, our Institute started a longitudinal study on the prevalence of chronic diseases in Croatia. The sample comprised urban and rural population from two continental and three coastal areas, with follow-ups in 1972 and 1982. The results showed marked regional differences in general and cardiovascular mortality. Mortality was the lowest in the coastal region (Island of Vis) and the highest in the northern, continental region (Virovitica—town and surroundings). Trace element deficiency is considered to be a risk factor for cardiovascular disorders. Therefore, during the fourth follow-up in 2006, serum concentrations of selenium (SSe), copper (SCu), and zinc (SZn) were additionally measured in 37 elderly subjects from Vis (20 women and 16 men, aged 72–87) and 63 subjects from Virovitica (38 women and 25 men, aged 71–88). The study was performed in accordance with the ethical principles of the Helsinki Declaration and approved by the Institute's Ethical Committee. Trace element concentrations were determined using electrothermal (SSe) or flame AAS (SZn and SCu). Accuracy was checked using reference material (BCR no. 637-639) and through regular participation in the UK TEQAS programme. The results showed a significantly lower SSe ($p=0.000$) in subjects from Virovitica, whose general health condition and perceived physical fitness was worse than in the subjects from Vis. There were no significant differences between the groups in SZn, SCu, age, BMI, or alcohol intake. However, there was a significant difference ($p<0.02$) in the type of alcohol consumed: subjects from Virovitica drank less wine, but more beer and spirits. Furthermore, they reported significantly lower consumption of fish and olive oil and higher consumption of salt, smoked meat, and coffee. Our results indicate that in addition to several well-known beneficial components of the Mediterranean-style diet, selenium may play a role in the protection from cardiovascular morbidity and mortality.

5.S02

Studies on molecular mechanisms in understanding copper-induced amyloid beta aggregation and its relevance to Alzheimer's disease

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Many therapeutic strategies have been tried to prevent Cu++-induced conformational changes and aggregation. We proposed to study the molecular events in Cu++-induced Abeta aggregation and its dissolution. Abeta (1–40) interaction studies were carried out by employing methods such as circular dichroism (CD), thioflavin-t binding and fluorescence-quenching measurements, transmission electron microscopy (TEM), polyacrylamide gel electrophoresis (PAGE), high-pressure liquid chromatography (HPLC), regression analysis and molecular modeling techniques. CD studies revealed that Abeta–Cu complex formed Beta sheet. TEM studies showed morphology of Abeta aggregation induced by Cu++. SDS-PAGE showed that chelator molecule stabilizes the monomer and prevents oligomerization. Thio-T fluorescence studies found metal chelator to be most effective at a molar ratio of 0.5:1 by incorporating a single dosage at a fixed time. Incorporation of multiple dosages at different time intervals did not work much effectively. Moreover, the regression analysis was used to understand binding patterns of chelator. Both soluble and aggregated Abeta were subjected to gel filtration analysis, wherein soluble Abeta gave a single peak in contrast to aggregated Abeta which revealed multiple peaks. On addition of chelator, the number of peaks were significantly reduced, leaving behind one major peak corresponding to soluble Abeta. The stoichiometry of binding is ~1.2 with a dissociation constant Kd of 5.3×10–4 M and an association constant Ka of 3.84×10–5 M as calculated by mass action plot. The above results were verified by geometry optimization of the chelator structure followed by molecular docking. The best ligand pose energy was calculated to be −9.4321 Kcal/mol at a net molecular charge of zero. Hence, we understood a new mechanism of Cu++-induced aggregation of Abeta, and prevention of fibrillation process by chelator.

Category 6: Fetal development and pediatric disorders

6.P01

Levels of toxic heavy metals and essential trace elements in maternal blood, cord blood, and placental tissues in Japanese pregnant women

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We have been performing a prospective cohort study to examine the effects of perinatal exposures to heavy metals and other chemicals on child development (Tohoku Study of Child Development: TSCD) in Japanese children. Mother–infant pairs were recruited in an urban area of Tohoku district, Japan. In this study, we focused to determine the maternal and fetal heavy metal levels such as copper (Cu), zinc (Zn), selenium (Se), cadmium (Cd), lead (Pb), arsenic (As), antimony (Sb), tin (Sn), and bismuth (Bi). Maternal peripheral blood, cord blood, and placenta were collected (n=578) for heavy metal analyses and samples were kept at −80°C until analyses. Heavy metal concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS). For undetected samples, a value of one half (1/2) of the detection limit was assigned. Concentration values were indicated as median. Cu concentrations were 1,284 ng/ml in maternal blood and 537 ng/ml in cord blood, those of Zn were 4,850 and 2,007 ng/ml, and those of Se were 180 and 192 ng/ml, respectively. Concentrations of Cd, Pb, and As in maternal blood were 1.16, 10.86, and 4.14 ng/ml, respectively. Cd was detected in 73% of all cord blood samples, with a median of 0.50 ng/ml. Pb was detected in 100% of all cord blood samples with a median of 9.90 ng/ml. Concentration of As in cord blood (detected in 99%) was 3.71 ng/ml. Concentrations of Zn, Cu, Pb, Cd and As in maternal blood were significantly higher than those of cord blood. Sb was detected in 72% of all cord blood samples; however, Sn and Bi were detected in only 44 and 7% of all maternal blood, 36 and 18% of all cord blood samples. In this study, it was found that Cd, Pb, and As were detected in maternal blood, cord blood, and placenta. However, Pb levels were lower than those of the previous reports in Japan and other countries. In spite of their use as industrial materials for Pb-free solder, exposures to Sb, Sn, and Bi were low.

6.P02

Impairment of iodine and thyroid hormone metabolism in very premature infants

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Iodine, as an irreplaceable part of thyroid hormones, belongs among the essential substances indispensable for the proper development of young mammals. The neonatal thyroid economy is extremely sensitive to fluctuations in the iodine supply from the mother. All preterm infants show transient hypothyroxinemia during the first 6–8 weeks of extrauterine life, and their future fate may be significantly influenced by the degree of hypothyroidism in the early postnatal period. However, in neonates, especially in very preterm infants, the relative contribution of peripheral tissues and the thyroid gland to the total production of circulating triiodothyronine (T3) has not yet been well defined. In this study, we present our observations on postnatal development of plasma thyroid hormones levels in normal and critically ill premature neonates. The main focus of this study was on very low birth weight newborns (470–1,370 g; 70% of neonates <1,000 g) with a high rate of mortality (20% of infants died within 1 month after delivery). Enzyme activities of all the three types of iodothyronine deiodinases were followed in autopsy samples from several tissues, to better characterize the relationships between peripheral metabolism of thyroid hormones and thyroid status in critically ill very premature infants. The results obtained support the view that peripheral tissues of very preterm infants are involved in local generation of T3 and inactivation of thyroid hormones, but contrary to tissues of adults and term-

born infants, do not represent a major source of circulating T3.

6.P03

Cadmium exposure and trace elements in breast milk

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Cadmium (Cd) is a widespread toxic contaminant that affects kidney function and bone metabolism even at environmental exposure levels. Recent studies indicate that Cd exerts both estrogen- and androgen-like effects. The general population is exposed to Cd mainly via food such as, cereals and vegetables, particularly rice, as Cd is easily taken up from soil. In general, women have higher Cd body burden than men, probably due to increased absorption at low iron stores and during pregnancy. Cd seems to be poorly transferred over the mammary gland, but experimental studies indicate neurobehavioral effects after exposure via breast milk, possibly related to the elevated absorption in infancy. In our ongoing studies, we are investigating the mechanisms of cadmium uptake and transport. These studies are nested into the Maternal and Infant Nutrition Interventions of Matlab (MINIMat), a food and micronutrient supplementation trial in Matlab, Bangladesh, including 4,500 women enrolled in early pregnancy and followed until 6 months postpartum. Concentrations of Cd in urine (median 0.59 μg/L, N=890) and blood (1.2 μg/kg, N=296) collected in early pregnancy showed that the Matlab women have elevated exposure, probably mainly via food, rice being the main staple food. Preliminary results indicate a positive association between Cd in blood and breast milk (rs=0.36). Median Cd concentration in breast milk was 0.14 μg/kg (10th and 90th percentile: <0.1 and 0.34 μg/kg, respectively N=199). Furthermore, there are significant associations between Cd and several essential trace elements in breast milk. Financial support provided by UNICEF, Swedish International Development Agency (Sida; SWE-2003-201A), USAID, DfID, and CHNRI are gratefully acknowledged.

6.P04

Copper and zinc status in the breast milk of mothers with Wilson's disease

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Female patients with Wilson's disease can become pregnant and give birth, at which time most of the mothers want to breastfeed their infants while continuing their treatment for Wilson disease. However, to the present time, copper concentrations in the breast milk of mothers with Wilson's disease have not been investigated. Here, we report on the copper and zinc status in the breast milk of mothers with Wilson's disease who are being treated with a chelating agent or zinc. Samples of breast milk were taken several times from four mothers with Wilson's disease. Two of the patients were being treated with trientine, a third patient was being treated with penicillamine, while the fourth patient was being treated sequentially with zinc and trientine. Copper and zinc concentrations in the breast milk were analyzed by atomic absorption spectrometry. The copper distribution was also analyzed by high-performance liquid chromatography (HPLC)/inductively coupled plasma mass spectroscopy (ICP-MS). The mean concentrations of copper and zinc in control breast milk samples were 28 and 123 μg/dl, respectively. The copper and zinc concentrations were within normal levels in the breast milk of mothers with Wilson's disease being treated with zinc. Of the three patients treated with trientine, copper concentrations in the breast milk were lower than controls in one patient, while zinc concentrations were lower than controls in two patients. In the case of the patient treated with

penicillamine, both copper and zinc concentrations were low. HPLC/ICP-MS results suggested that copper in the control breast milk is bound with ceruloplasmin and albumin. In the breast milk of patients treated with trientine, however, the binding of copper to trientine was also detected, suggesting that trientine is secreted into the breast milk.

6.P05

Analysis of some important trace elements in the placentas of healthy and preeclamptic Turkish women in the province of Kayseri-Turkey

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During pregnancy, trace elements are indispensable for life maintenance not only for the mother but also for the fetus. Some trace elements are considered essential as they are needed for the life process, whereas others are considered highly toxic. The human placenta facilitates the passage of some bio-substances to the fetus while at the same time acts as a barrier to other materials. While nutrient substances cross the placenta barrier, there is also a possibility that some harmful substances may pass through the placenta and can damage the embryo. In this study; six trace elements were determined in placenta tissues of control and preeclampsia patients in the Kayseri province. Approximately 400 mg placenta samples were digested with 9 mL of 68% ultrapure nitric acid and 1 ml of 35% hydrogen peroxide using a microwave digester. Completely clear, colorless, homogenous digests were obtained and subsequently diluted with high-purity water to 30 ml. The diluted solutions were supplied to the determination of six trace elements, Zn, Ni, Cu, Cd, Pb, Fe, by ICP-OES. The average range of concentrations for trace elements in placenta based on wet weight were found to be (μmol/g, based on wet weight); Zn: 0.048–0.55, Ni:0.08–1.67, Fe: 0.032–2.65, Pb: 0.048–1.58, Cu: 0.031–5.5, Cd: 0.056–9.75; and Zn: 0.018–0.17, Ni: 0.02–3.68, Fe: 0.18–7.5, Pb: 0.034–3.47, Cu:0.49–2.73, Cd: 0.045–1.08, for control and preeclampsia patients; respectively. From the results, there was no considerable statistical correlation between the concentration of trace elements observed between the control and pre-eclampsia patients.

6.P06

Effect of 1 year of iron/zinc fortification on lead levels among children aged 1–4 years

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Iron deficiency and lead toxicity are detrimental in early development. Iron deficiency is associated with lead toxicity due to increased lead absorption among iron-deficient children. Study in Bangalore showed that iron fortification reduced lead levels among lead exposed, iron deficient children. We evaluated the effect of zinc and iron fortification of milk on blood lead levels and hematological markers among children aged 1–4 years. In a community-based, double-masked, RCT, a total of 633 children were enrolled and randomly allocated to fortified milk (Mn, n=316) or same milk without fortification (Co, n=317) for a period of 1 year. Fortified milk delivered 9.6 mg zinc, 9.6 mg iron, 6.6 μg selenium, 0.3 mg copper, 1,110 IU vitamin A, 48 mg vitamin C, 8.1 mg vitamin E per day. Venous blood sample was collected at baseline and at end study. Blood lead levels were estimated using atomic absorption spectrophotometer with a graphite furnace (Perkin Elmer Analyst 800) at end study. ZnPP levels which have been previously used as a screening tool for lead levels showed that both the groups were similar at baseline [Znpp (μmol/g of heme) >200 Mn: 41.7%, Co: 40.8%]. Fortification significantly improved the markers of iron status and reduced the prevalence of iron deficiency in the fortified group compared to the non-fortified group (previously reported).There was a significant decrease in mean blood lead concentration (μg/dL) in the fortified group compared to the control group [Mn:16.69±6.30, Co: 19.52±7.51; Diff of mean (95% CI) −2.84 (−4.15, −1.52), p<0.001]. The proportion of children with high blood levels (>21.4 μg/dL) was lower in the fortified group (Mn: 16.7%, Co: 35.6%). Our findings suggest that consumption of iron- and zinc-fortified

milk in addition to significant improvement in iron status and anemia also benefit by reducing blood lead levels among children (study registration number NCT00255385).

6.P07

Nutritional value of processed vs traditional snacks, consumed by children, in relation to their Zn and Cu content

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Nutritional status and profile of school aged children and adolescents is nowadays widely affected by snack foods replacing proper daily meals. Zinc and copper bioavailability is significantly related to young people's normal nutritional status and growth, while Zn and Cu deficiency is associated to developmental deficits. Aim of the present research is to find the nutritious foods among several snack foods, abundantly consumed by school pupils, in relation to their Zn and Cu content. Thorough investigation over the products offered in school buffets revealed an abundant variety of industrially processed snack foods and a very limited number of more or less natural and traditionally prepared ones. Furthermore, the processed snacks were found to overflow the supermarket shelves and the media advertising time. Three groups of 50 snack products of various commercial origin were analyzed by AAS for their zinc and copper content: (1) bakery products, (2) sugar products, and (3) crispy products. Each group comprised of two subgroups: (A) processed products, (B) less processed, traditional products. Results show that Zn and Cu content, either per packing or per kcal of the product, are significantly higher in the less processed snacks (B subgroups). Indicatively, mean value±SD of Zn (μg)/product kcal were: 1A: 2.05±0.94, 1B: 5.62±1.75.Less processed snacks (B subgroups) offer also more essential metals (Zn, Cu) and less kcal per packing compared to highly processed (A subgroups), e.g., 2A: mini strudel Kcal/packing: 477.36, Zn μg/packing: 536.77, Cu μg /packing: 100.65, 2B: pasteli kcal/packing:237.60, Zn μg/packing: 1,980.00, Cu μg /packing: 576.00. It is concluded that nutritious, anti-obesity key foods may be the less processed snacks providing, closer to dietary recommendations for school aged children, the essential metals Zn, Cu (supported by the: EC E.P.E.A.E.K. II "HRAKLEITOS" (61/1783) project).

6.P08

Exposure assessment of methylmercury in the Japanese pregnant women

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Methylmercury (MeHg) is an important environmental neurotoxicant that is present in seafood and affects the developing and mature nervous system. We have been performing a cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of prenatal exposures to MeHg. Our objective in the present study is (1) to show exposure measurements such as mercury concentrations in maternal blood, cord blood, and maternal hair, (2) to examine the relationships of mercury concentrations between those materials. We recruited approximately 600 pregnant women. Maternal blood and maternal hair were collected perinatally. Total mercury analysis was carried out by cold vapor atomic absorption spectrometry (CVAAS). MeHg was determined by gas chromatography with electron capture detection (GC-ECD). As the result, geometric mean of total mercury concentration (and MeHg) of maternal blood was 5.4 ng/g (5.1 ng/g), cord blood was 9.9 ng/g (9.5 ng/g), maternal hair from scalp to 3 cm was 2.0 μg/g. In previous report of WHO (1976), the hair/blood ratio is about 250 as

determined by linear regression. However, in this study, the ratio was about 320 in pregnant women presumably due to hemodilution. The hair/blood ratio was found to be higher at lower mercury concentrations. This finding suggests that the hair/blood ratio of pregnant women differs from those of nonpregnant women and men, and the ratio may be affected by mercury concentration in blood.

6.P09

Iodine deficiency disorders in Marrakech, Morocco

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Micronutrient malnutrition, insufficient dietary intake of nutrients such as iodine, affects the health and survival of more than 2 billion people worldwide. Women and children are most at risk. Endemic goiter, which reflects iodine deficiency disorders (IDD) in a population, is localized in the Rif and Atlas mountains affecting the Moroccan population living in these areas. Iodine deficiency impairs physical and mental development including intellectual capacity. The presence of goiter (enlargement of the thyroid gland) reflects significant iodine deficiency in a population. An epidemiological survey of iodine deficiency disorders conducted in two regions of Marrakech in 2003–2004 involving 1,544 subjects aged 10–50 years showed a goiter prevalence of 38% in the Ourika area and 30% in the Tahanouate area. Urinary iodine excretion indicated severe (21%), mild (24%), and moderate (18%) iodine deficiency in the mountain population. Goiter prevalence was higher among females than males. This study found urinary iodine levels among goitrous and non-goitrous individuals from endemic Marrakech to be lower compared to those of individuals from the non-endemic region of Casablanca. Moreover, analysis of the iodine content of cereals, drinking water, sea and rock salt, and cow and breast milk was found to be low. Results found goiter prevalence among children to be 28%. More than 21% of 281 urine samples showed severe iodine deficiency. Cretinism was also present in some endemic areas. The data collected during this survey provides support for the salt iodation campaign currently in progress in Morocco.

6.S01

Increased cadmium absorption in iron-supplemented suckling piglets

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There are indications that newborns are more susceptible to cadmium toxicity than adults and it is therefore important to minimize the cadmium exposure at young age. Cadmium is believed to be absorbed from the intestine by the iron transporters DMT1 and FPN1, and there are indications that also MT is involved. In adolescents and adults, low-iron status is known to increase the uptake of cadmium. In the present study, suckling piglets were used to examine whether iron status influences cadmium absorption in newborns and the possible roles of DMT1 and FPN1, and MT in this process. An oral cadmium dose (20 μg Cd/kg body weight) was given daily for six consecutive days on postnatal days (PNDs) 10–15 to iron-supplemented and iron-deficient piglets. Cadmium levels in blood and kidneys were measured on PND 16 by ICP-MS as indicators of cadmium absorption. A statistically significant increased growth, which was not affected by cadmium, was observed in iron-supplemented piglets. In contrast to what was expected, higher cadmium absorption was observed in the iron-supplemented piglets compared to the iron-deficient ones. In kidneys, the cadmium concentration was increased twofold in the iron-supplemented compared to the iron-deficient piglets. Median cadmium levels in blood were also higher in iron-supplemented piglets, although not statistically significant. However, a positive significant correlation between cadmium in blood and kidneys were seen. Localization and quantitative expression of duodenal DMT1, FPN1 and MT were not affected by iron status, indicating that the increased cadmium absorption in the suckling newborn after iron supplementation is due to other mechanisms. In conclusion, our results indicate that

iron supplementation to suckling newborns increases cadmium uptake and that the investigated transporters are not involved in the process at this young age.

6.S02

Red-cell trace minerals in children with autism

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Abnormalities in mineral-dependent antioxidant enzymes in children with autism raise interest in the determination of trace mineral status in this population. A cross-sectional investigation of red cell mineral levels was carried out among 20 children with autism and 15 controls. Children with autism demonstrated significantly lower red cell selenium ($p<0.0006$) and higher molybdenum ($p<0.01$) than the controls. There was a trend toward lower red cell zinc, and higher cobalt and vanadium, among the children with autism. There were no differences in red cell levels of chromium, copper, manganese, or magnesium. These findings confirm an earlier report of low red cell selenium in autism and support a role for decreased trace mineral status in oxidative stress in autism through alteration of selenium-dependent antioxidant enzymes and increased lipid peroxidation.

6.S03

Consumption of milk fortified with iron, zinc, and trace amounts of copper for 1 year: effects on copper status among preschool children

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With demonstrated benefits of supplemental zinc on morbidities, most developing countries may initiate national programs to provide additional iron and zinc to young children. However, there are concerns that supplemental iron or zinc can interfere with the copper status. It is not clear if supplementing iron and zinc for a prolonged duration has any effect on the copper status among young children. In a randomized, double-masked, community-based trial among 1- to 3-year-old children, we compared the effects of daily consumption of fortified milk (Mn; providing 10 mg/day, zinc 10 mg/day, and copper 0.3 mg/day) to non-fortified milk (Co) for 1 year, on copper status. Venous blood samples were drawn at enrollment and after 1 year of supplementation. Blood samples were run through the coulter counter for hematological counts, and serum copper (SCu) levels were estimated in triplicate using AAS with graphite furnace platform. Children in both groups (Mn: $n=122$; Co: $n=121$) were comparable for baseline characteristics, including SCu levels. Significant improvement in iron stores and anemia has been reported elsewhere. Neutropenia typically associated with copper deficiency was not observed. Compared to their baseline levels, the mean SCu levels among children in Co group increased by 16.96 μg/dL (SD: ±50.42) and that for Mn group showed only a modest increase of 3.97 μg/dL (SD: ±43.27). At end-study, although compared to children in Co group, children in Mn group on an average had SCu levels lower by 7.89 μg/dL (95% CI: 2.42–13.53; $p=0.005$), Mn group had significantly lower proportion of copper-deficient children defined as SCu levels <70 μg/dL (Mn: 3.2 vs Co: 9.1%; $p=0.05$].The study provides evidence that adding trace amounts of copper to vehicles used for long-term provision of iron and zinc does not result in clinical or subclinical copper deficiency and, on the other hand, resulted in lowering of high levels of copper rather than causing elevation.

Category 7: Infectious diseases and immune disorders

7.P01

Tissue uptake of mercury is changed during the course of a common viral infection in mice

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Mercury (Hg) has been shown to have immunotoxic effects and to influence the severity of infection. However, the impact of infection on the normal Hg homeostasis in different organs involved in the disease process has not been studied. In this study, Hg was measured through inductively coupled plasma-mass spectrometry (ICP-MS) in the intestine, serum, liver, and brain on days 3, 6, and 9 of coxsackievirus B3 (CVB3) infection in female Balb/c mice. The severity of the infection was assessed from clinical signs of disease and the number of virus particles in infected organs. CVB3 and gene expression of metallothionein 1 (MT1) were measured by reverse transcription polymerase chain reaction (RT-PCR). Gene expression of MT1 increased and peaked on day 3 in the brain and liver and on day 6 in the intestine. This peak in MT1 in the liver and brain corresponded to the peak in virus numbers in these tissues. Furthermore, Hg increased on day 6 in the brain but went unchanged in the liver, whereas in the intestine and serum, Hg tended to decrease on all days of infection. The maximum decrease, in comparison with non-infected mice, occurred in the intestine on day 9 and in serum on day 6. An infection-induced increase of Hg in the brain may be due to the peak of virus replication and the associated infection-induced expression of MT1. Moreover, the decrease of Hg in serum and the intestine but a concomitant intestinal increase in MT1 on day 6 may reflect a flux and increased retention of Hg to infected organs such as the brain. The pathophysiological interpretation of these findings requires further research.

7.P02

Zinc treatment prevents dysmenorrhea

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More than half and by some estimates 90% of women experience menstrual cramps during the first several days of menstruation. Although the uterus contracts and relaxes routinely, during menstruation, the contractions are much stronger producing pain and "cramps". Women with dysmenorrhea have high levels of prostaglandins, hormones believed to cause menstrual cramping. Prostaglandins are believed to temporarily reduce or stop blood supply to the uterus, thus depriving the uterus of oxygen resulting in contractions and pain. One would expect zinc, like the nonsteroidal anti-inflammatory drugs used to treat cramping, to reduce the production of prostaglandins. Zinc inhibits the metabolism of prostaglandins ruling out this mechanism of action, suggesting erroneously that zinc deficiency would prevent cramping. However, it is shown by case histories that zinc, in one to three 30-mg doses given daily for 1 to 4 days before onset of menses, prevents essentially, all in all, warning of menses and all menstrual cramping. One hypothesis for a mechanism of action is that a precursor (COX-2) or metabolite of prostaglandins causes menstrual cramping and not prostaglandins themselves. Another hypothesis is that zinc has antioxidant and anti-inflammatory actions in the uterus. Improvement in microvessel circulation by zinc may help prevent cramping and pain. In patients consuming 31 mg of zinc per day, premenstrual tension (PMT) symptoms did not occur, while in patients consuming 15 mg of zinc, PMT symptoms did occur ($p<0.001$). Protocols using 30 mg of zinc once to three times a day for 1 to 4 days immediately before menses to prevent dysmenorrhea are described, and they are recommended for additional study. The side effect from the absence of all warning of pending menses due to zinc treatment was concern of possible pregnancy. The United States RDA for zinc appears to be too low to optimize women's health and prevent menstrual cramps.

7.P03

Evaluation of the oxidant/antioxidant status in patients with Behçet's disease

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Behçet's disease (BD), a multisystemic inflammatory chronic disease, is a vasculitis that can affect vessels of all sizes. Various mechanisms (infectious, genetic, and immunological) could initiate BD, but its exact pathogenesis still remains uncertain. An oxidative stress state resulting in free radical excess would be also implicated in BD patients in tissue lesions

genesis. Our purpose was to prospectively study the oxidative status and antioxidant defences in patients with BD in comparison to a healthy control group. We prospectively enrolled 40 patients with BD (27 men, 13 women, mean age: 38.8 years) and 40 age and sex-matched healthy control subjects. BD patients, ageing more than 20 years and meeting the International Study Group (ISG) of BD criteria, were included in the study. In both groups, we studied the malonylaldialdehyde (MDA), a product of lipid peroxidation, the glutathione in its oxidized (GSSG) and reduced forms (GSH), and erythrocyte antioxidant enzymes: the Cu–Zn superoxide dismutase (SOD), the glutathione peroxidase (GSH-Px) and the catalase. When compared to controls, mean MDA level was significantly increased ($p<0.05$) in BD patients. An increase of oxidized glutathione (GSSG) and a decrease of the GSH/GSSG ratio were noticed in the same group ($p<0.001$). The activity of SOD was significantly lower in BD patients ($p<0.001$). On the other hand, catalase activity was increased ($p<0.001$) and also GSH-Px but without significance. An imbalance in the oxidant status is thus observed in BD patients. In BD, all functions of neutrophils are activated and so is their production of reactive oxygen species. Antioxidant defenses would first increase their activity trying to overcome the FR excess aggression then, overburdened, would probably fail. A rational strengthening of antioxidant defenses in BD patients should be, in the future, part of an optimal treatment strategy.

7.P04

Anemia, iron deficiency, and parasitism in children from a rural community of Venezuela

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The World Health Organization estimates that 2 billion people in the world suffer from anemia, principally caused by iron deficiency and often linked to intestinal parasitic infection. The most affected populations are in rural and marginal urban areas of developing countries. This study determined the prevalence of anemia, iron deficiency, and intestinal parasitosis in a random population of children under 15 years of age in the "La Bucarita" rural community of the Andrés Eloy Blanco Municipality, Lara State, Venezuela. The random sample consisted of 31 children <2 years, 166 children 2–6 years, and 204 children 7–14 years. Values for four indicators of iron status are given: hemoglobin concentration, serum ferritin concentration, serum iron, and total iron-binding capacity (TIBC). The results showed anemia to be present in 17.2% of the sample and 31.9% with iron deficiency (33.6% with anemia, 66.4% without). Parasitosis was present in 79% of the children. The most common parasite was *Ascaris lumbricoides* (51.7%), followed by *Trichuris trichiura* (42.8%). The youngest group was the most affected by iron deficiency and the 7- to 14-year group the most affected by parasites. There is an inverse correlation between weight, size, and age with the prevalence of anemia. There is no significant correlation between parasitosis and anemia. Iron deficiency, anemia, and parasite infection evidently represent an important health problem in this population, requiring prompt attention.

7.P05

Zinc and copper in red blood cells, lipid peroxidation, nutritional status, and inflammatory response in the progression of chronic hepatitis c virus infection

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Oxidative damage may play a role on progression of liver injury in patients affected by chronic hepatitis C

virus infection. Some trace elements, as Cu and Zn, which are involved in antioxidative enzymatic systems, may keep a relation with the activity of these enzymes. In addition, chronic virus C infection triggers cytokine activation, which in turn promotes enhanced lipid peroxidation; as oxidative damage causes cytokine release, a positive vicious circle ensues. Impaired nutrition may accelerate progression to cirrhosis. Based on these facts, we analyze in the present study the relationship between nutritional status, severity of liver disease (from biochemical and histological points of view), glutathione peroxidase (GPX) and superoxide-dismutase (SOD) activities, red cell Zn and CU contents, serum malondialdehyde (MDA) levels, and serum levels of different cytokines and antioxidants as vitamin A, C, and E in 66 patients affected by chronic hepatitis C virus infection, who received treatment with interferon and ribavirin for 48 weeks. Among several other results, we found an inverse relation between red blood cells Zn and MDA levels (rho=−0.40, p=0.005), but a direct one with nutritional parameters such as brachial perimeter (r=0.34) and waist circumference (r=0.30, p<0.05 in both cases), an inverse relation between cytokine levels and antioxidants, and a direct one, between lipid peroxidation and inflammatory response. Red blood cell ZN and GPX activity at admission were related to a positive therapeutic response, defined as negative viral replication 6 months after cessation of therapy. Our results stress the importance of oxidative damage and antioxidant systems in the progression of hepatitis C virus infection.

7.P06

Influence of toxic and essential metals on markers of atopy and ventilatory function in men and women

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The combined influence of age, smoking, alcohol, erythrocyte superoxide dismutase (SOD), glutathione peroxidase (GPx) in whole blood, blood lead (BPb), and cadmium (BCd) levels, and serum levels of copper (SCu), zinc (SZn), and selenium (SSe) on atopic status and ventilatory function was examined in the groups of men and women with no occupational exposure to metals. Markers of atopy included serum total IgE, skin prick test (SPT) to common inhalatory allergens, nonspecific nasal reactivity (NR) and nonspecific bronchial reactivity (BR). Parameters of ventilatory function included forced vital capacity (FVC) and forced expiratory volume in one second (FEV1), each expressed as percentage. A significantly (p<0.05) higher BPb, SZn, IgE, and incidence of positive SPT and lower SCu and NR was found in men than in women. In men, IgE inversely correlated with SSe (p<0.03), and FVC% and FEV1% inversely correlated with smoking intensity (p<0.0001 and p<0.005, respectively) and BCd (p<0.007 and p<0.005, respectively). In women, IgE positively correlated with BPb (p<0.02), and NR positively correlated with GPx (p<0.02). After adjusting for potentially confounding variables by multiple regression in the group of men, an increase in BR was associated with an increase in age (p<0.01) and a decrease in SSe (p<0.01) and log BPb (p<0.02); a decrease in FVC% was associated with an increase in smoking intensity (p<0.0002) and a decrease in SZn (p<0.05); a decrease in FEV1% was associated with an increase in smoking intensity (p<0.002) and SOD (p<0.05), and a decrease in SZn (p=0.05). In women, an increase in log IgE was associated with a decrease in SCu (p<0.05); an increase in NR was associated with an increase in GPx (p<0.02) and a decrease in SCu (p<0.05). The observed difference in response between the groups of men and women may partly be explained by different levels of relevant toxic and essential metals and particularly their combination.

7.P07

Can leg disorders of turkeys be prevented by some effective ingredients containing the feed additive Immunovet-HBMTM?

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Fast-growing turkey hybrids have been developed, a big breast being a valuable part of the body. The advantages of intensive body weight gain is often accompanied by leg disorders traced back to the disharmony between development of skeletal organs, bones, cartilages, tendons, and skin and the striated

musculature. Birds are restricted in their moving, their feed and water consumption is limited, while their body weight gain, their mortality and feed conversion rate is increased, as the profitability of rearing. In the etiology of leg disorders, deficiency of some vitamins and trace elements (Zn, Mn, Cu, etc.) play well-defined roles. The assessments of fast-growing animals by trace elements are nearly identical using a well-composed feed and taking into consideration of numerous factors influencing their absorption rate depending on the health status of birds. As the AGP-s is prohibited in EU, the composition of gut microflora can be regulated only by probiotics, probiotics, etc. maintaining the right ecosystem of gut flora assuring the normal absorption of nutritive materials. Immunovet HBM-TM is a prebiotic serving as substratum for the right formation of useful micro flora in the gut. It contains specific amino acids, vitamins, and trace elements. Immunovet HBM-TM has also antioxidants, immune-regulating and roborant effects. Immunovet-HBM™ added to the feed of commercial turkeys continuously increased their body weight gain, decreased the feed conversion rate, mortality, and leg disorders, compared to the control flocks. A clear relationship may be observed between the employing of Immunovet HBM-TM and the immune status of turkeys. The beneficial effects may be explained by the better immune-protection of epithelial lining of gut, the physiological absorption of nutrients including trace elements and vitamins, participating in prevention of leg disorders.

7.P08

Influence of selenium and *Eimeria tenella* infection on antioxidant status in chicks: status and resistance against infections

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The effect of selenium–yeast (Sel-plex 50, Alltech) on the antioxidant defense system (vitamins E, C, A, and Se content and Se-GPx-activity) in chicks experimentally infected with *Eimeria tenella* (Protozoa) was studied by us. The study was performed on 100 broiler chicks divided into four groups: first group—control, second—control and supplemented with Sel-plex, third group—infected with *E. tenella*, and fourth group—infected with *E. tenella* and supplemented with Sel-Plex. The chicks were fed a standard diet without antibiotics and coccidiostats. The second and fourth groups were fed on the diet to which Sel-Plex 50 was added—0.3 mg/kg diet. The third and fourth groups were infected with 10.8^4 sporulated *E. tenella* oocysts. Serum and liver Se content was determined by a fluorimetric method. The content of liver vitamins C, E, and A was established by HPLC systems. Serum Se-GPx activity was determined spectrophotometrically. An antioxidant imbalance was developed due to the *E. tenella* infection. It was expressed by reduced vitamins A, C, and E levels, and Se content and reduced GPx activity. The Sel-Plex administration almost restored vitamin E and Se losses and increased GPx activity in the infected chickens. The observed changes in the small intestine, lesions, and oocyst indices, body weight gain, and feed conversion ratio were indicative of a severe *E. tenella* infection. They were correlated with oxidative stress. Administration of Sel-Plex resulted in significant increase in weight gain, improved feed conversion ratios, and increased GPx activity, Se, and vitamin E liver level in the control and infected chicks. The oocyst and lesion indices were slightly reduced after the treatment in the infected chicks. The experiment was approved by the Committee on Animal Experimentation at Trakia University, Stara Zagora, Bulgaria and was performed according to the recommendations of the Directive 86/609/EC.

7.S01

Effects of cocktail antioxidant supplementation on oxidative stress in aids

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Infection of HIV/AIDS is a worldwide problem of increasing magnitude. Epidemiological studies have shown an inverse relationship between antioxidant intake and the incidence of the disease. Nutritional problems in patients with HIV/AIDS may be due to several mechanisms working independently or synergistically. The most effective role the clinician can play in the nutritional care of these patients is close surveillance of nutritional complications over time and with evolving medical therapy. Twenty-four Latvian male volunteers were HIV-infected outpatients (age 35, 3±0.5). None of the screened subjects had C4 T cell counts less than 200 cells in microliter of blood, none had active opportunistic infections or malignancies, and all were readily mobile. Ten uninfected control males were similar in age to the HIV-infected group. HIV-infected subjects were treated additionally with Coenzyme Q10 (CoQ10), L-Carnitine 500 mg/day, Bio-Selenium + Zinc that contains: A-vitamin 1,000 μg, B6-vitamin 2.2 mg, Vitamin C 90 mg, Vitamin E 15 mg, Zinc 15 mg, and selenium (Organic) 100 μg, each for 6 weeks. Significant decrease of fMLP-stimulated PMNL chemiluminescence ($p<0.05$) confirms the antioxidative properties. Serum concentration of selenium increased after CoQ10 treatment. Monitoring antioxidant enzymes and other metabolic changes in patients with HIV infection is recommended. The supplementation of antioxidants is worth applying to HIV patients.

7.S02

Current clinical practices for trace element supplementation in French burn centers

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Severely burned patients need a particularly heavy resuscitation treatment in a specialized burn center (BC). In case of thermal injury, the oxidative stress increases the production of free radicals, whereas the antioxidant defenses collapse, generating deleterious tissue damages. As the trace elements are known to be involved in antioxidant defense, immunity and cicatrization, their supplementation should be taken into account in the entire nutritional status of the burned patients. To evaluate the place of these micronutrients in the therapeutic constant care of severe burned patients, we choose to analyze the current clinical practices of the trace element supplementation by submitting a survey to the 24 French specialized BC. The BC participation rate to our survey reflects the interest of the medical practitioners in the supplementation of severe burned patients, but it underlines that trace element supplementation is still often too limited according to the expected clinical benefit in relation with the previous published studies. The professional practices differ from each other and generally remain quantitatively too restricted for responding to the severe burned patient specific needs. Complementary workshops are necessary to define consensual practices and to lead the pharmaceutical laboratories to adapt the patent medicines to the specific needs of the BC patients.

7.S03

Withdrawn

7.S04

Iron homeostasis is changed in *Chlamydophila pneumoniae*-infected tissues

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Chlamydophila pneumoniae (*C. pneumoniae*) might be involved in the pathogenesis of some chronic conditions including atherosclerosis. Like many bacteria, *C. pneumoniae* need iron (Fe), but it is not known if infections affect gastrointestinal uptake and uptake in infected tissues of trace elements. Consistent responses during infection are a decrease in plasma levels of iron (Fe) and zinc (Zn) and an increase in copper (Cu). The aim of this work is to study changes of Fe in serum, heart, liver, and intestine during *C. pneumoniae* infection and if these are associated with changes in bacterial replication. Adult female C57BL/6J mice were divided into four groups; six mice in each group. Three groups were infected with *C. pneumoniae*, and one group was

sham-inoculated with SPG. On days 2, 5, and 8, six infected and two sham-inoculated mice were sacrificed. Whole blood and tissue samples from heart, liver, and intestine were collected. Fe in tissues and Fe and Cu/Zn ratio in serum were measured by inductively coupled plasma mass-spectrometry (ICP-MS). Bacterial DNA in blood, heart, and liver and bacterial mRNA in liver were measured with real-time PCR. Clinical signs of disease and serum Cu/Zn ratio peaked on day 5. During the infection, Fe decreased in serum, increased in the liver, tended to increase in the heart, but was unchanged in the intestine. In the blood, *C. pneumoniae* was only detected on day 2. Bacterial DNA peaked on day 2 in the heart and on day 5 in the liver, but the number of mice positive for *C. pneumoniae* in the liver was highest on day 8, with all mice positive. Viable bacteria in the liver was highest on day 8, which was associated with an increased Fe concentration in the liver. The pattern of infection-induced changes in Fe in all studied compartments support the hypothesis that Fe is pivotal for bacterial growth. It remains to be shown whether Fe has a role in the development of chronic *C. pneumoniae* infection.

7.S05

Adding iron to zinc reduced the efficacy of zinc on serious adverse health outcomes among preschool children in malaria endemic island of Pemba

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Zinc (Zn) and iron (Fe) deficiency often coexist among young children in low income countries, and so, combined Zn and Fe supplementation has been proposed. Iron may inhibit Zn absorption but that should lead to an additive interaction. In a four-celled study, where two arms allocated to receive supplemental iron and folic acid were stopped by the DSMB due to overwhelming evidence of increased adverse events, we evaluated interaction between zinc and iron in terms of effect on adverse outcomes (hospitalizations and deaths). Compared to the consumption of Fe/FA with Zn, consumption of Zn resulted in a significant reduction in relative risk of adverse events by 16% (95% CI: 7 to 24%; $p=0.001$) and reduction in mortality by 19% (95% CI −3 to 37%; $p=0.08$). Compared to placebo, children consuming Fe/FA showed a nonsignificant increase in adverse events 10% (95% CI: 0 to 22%; $p=0.09$) and deaths 17% (95% CI −8 to 48%; $p=0.19$). Our data suggests that in settings with high malaria transmission, there may be a significant interaction between giving iron and zinc together even at a molar ratio of 1:1, which is above and beyond additive interaction and needs to be investigated further.

Category 8: Molecular nutrition for the clinician

8.P01

The effect of selenium supplementation to hemodialysis patients on plasma glutathione peroxidase (gsh-px) activity and gsh-px protein level

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Numerous authors have shown that Se concentration and GSH-Px activity in plasma of chronic disease patients (CKD) and patients on hemodialysis (HD) are lower than in healthy people, but there are few publications on the level of GSH-Px protein in those patients and no reports on the effect of Se supplementation to HD patients on the level of this enzyme. Se concentration in plasma was carried out in a group of 40 nondialyzed CKD patients, 11 HD patients supplemented with 200 μg Se/day for 3 months, 9 HD patients administered with placebo, and 40 healthy persons. Se was measured by graphite furnace atomic absorption spectrometry, plasma GSH-Px activity was determined by the spectrophotometric method, and GSH-Px protein level by the sandwich ELISA method using polyclonal antibody specific for

human plasma GSH-Px. Se concentration, plasma GSH-Px activity, and the enzyme protein level were significantly lower in CKD and HD patients. Se supplementation to HD patients induced a moderate increase in plasma enzyme activity but had no effect on GSH-Px protein level. The reduced activity and level of the enzyme was not due to Se deficiency. A lower synthesis of plasma GSH-Px means that the damaged kidney of CKD patients is not able to synthesize GSH-Px, even after induction with selenium. As activity and level of plasma GSH-Px are always significantly lower in CKD patients compared with healthy individuals, the measurement of the activity and the protein level of this enzyme may prove to be an additional biomarker useful in the diagnosis of kidney damage in this group of patients.

8.P02

Molecular diagnosis for atypical patients and carriers with Wilson disease

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Wilson disease is a genetic disorder of copper metabolism characterized by hepatic and/or neurological manifestations. This disease is caused by mutations in the gene of copper-transporting ATPase (ATP7B). Wilson disease is a treatable disorder. Treatment involves the removal of excess copper by chelating agents (D-penicillamine or trienthine 2HCl), and/or blocking intestinal copper absorption by oral administration of zinc salt. Early diagnosis is very important to improve the prognosis of this disease. However, biochemical studies are not sufficiently effective for the definitive diagnosis of young patients, atypical cases, and carriers. This study presents the molecular diagnosis of patients and carriers of Wilson disease. We analyzed four patients and three of their families. Genomic DNA was isolated from peripheral blood leukocytes of patients and their family. All exons of the ATP7B gene were amplified by genomic polymerase chain reaction (PCR), and then analyzed by direct sequencing. All of gene analysis was performed under written informed consent. Two patients, one infant, one carrier, and two atypical cases were diagnosed by ATP7B gene analysis. We conclude that the molecular diagnosis of Wilson disease is very useful for the identification of young patients, atypical cases, and familial analysis. When ATP7B gene mutations of patients are detected, their familial analyses with ATP7B gene analysis are very easy and definitive to identify patients, carriers, and healthy persons.

8.P03

Role of Zn in ropivacaine and neutral endopeptidase: toxic effects on human ceratinocyte cells

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Neutral endopeptidase (NEP/CALLA/CD10/Neprilysin E.C. 3.4.24.11.) is a Zn-dependent metallopeptidase, inhibiting the analgesic effect of enkephalins, while expression and overexpression of NEP results in growth inhibition, induction of apoptosis, and promotion of apoptosis by toxic compounds. Ropivacaine is a local anesthetic inhibiting proliferation and inducing apoptosis on human keratinocytes HaCaT. Zinc (Zn) is an essential metal participating in various vital cellular functions. The aim of the present study is to investigate Zn effects on ropivacaine-mediated growth and viability reduction, apoptosis induction, and NEP downregulation in human skin keratinocytes. Immortalized human skin keratinocytes (HaCaT) were transfected either with the expression vector pcDNA3.1B, currying the neo-resistant gene or pRc/CMV-NEP carrying also the rabbit NEP cDNA via the DMSO-polybrene method. The transfected cells were selected in G418 for 2–3 weeks, and then subconfluent HaCaT, HaCaTneo, and HaCaTNEP were cultured in serum-free growth medium and were treated with ropivacaine (0–5 mM) with the addition of 15 μM Zn (Zn(NO3)2) for 48 h. Cell proliferation, viability, DNA fragmentation, and protein expression were processed. Zn (15 μM) inhibited cell growth and viability reduction and apoptosis induction by ropivacaine (0.5 and 1 mM) possibly by direct or indirect inhibition of caspase activation, in HaCaT cells. Ropivacaine (1–5 mM) downregulated the protein expression of NEP in HaCaT cells indicating that its

analgesic effect may be exerted via the release of enkephalin action. NEP overexpression caused reduction of cell growth and viability and also induction of apoptosis which was reinforced by ropivacaine (0.5–5 mM) and was not inhibited by Zn (15 μM), in HaCaT cells. Zinc may play a protective role against toxic effects expressed by exogenous or endogenous factors involved in pain pathways of the human body.

8.P04

Strong induction of arachidonate 12-lipoxygenase (alox15) in the intestine of iron-deficient rats results in the production of biologically active lipid mediators

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To identify novel genes associated with iron homeostasis, we performed gene chip analyses (Affymetrix) with intestinal mucosa from iron-deficient rats and from rats with a mutation in the principal intestinal iron transporter Dmt1 (Belgrade [b] rats). We hypothesized that genes that were induced in both models of Fe deficiency would likely encode proteins involved in the physiological response to iron deprivation. Computational analysis of the gene chip data and clustering algorithms identified a large group of similarly induced genes; gene ontology analysis revealed statistical enrichment of genes related to lipid metabolism. Most striking was induction of arachidonate 12-lipoxygenase (Alox15) in the duodenum and jejunum of Belgrade rats. Alox15 metabolizes polyunsaturated fatty acids (PUFAs) to biologically active lipid mediators. qRT-PCR studies demonstrated induction of Alox15 throughout the length of the intestine and also in the liver of iron-deficient and b/b rats. Immunolocalization studies showed expression of Alox15 in intestinal and colonic epithelial cells with much stronger expression in the iron-deficient rats. We also noted greatly increased hydroxy and hydroperoxy PUFA levels in the iron-deficient intestine, indicative of lipid peroxidation; the overall pattern of lipid peroxide products was consistent with Alox15 activity. We have thus documented significant regulation of Alox15 by iron in the mammalian small intestine and concomitant alterations in lipid homeostasis. These findings may be of significance in relation to increased villus surface area and crypt depth previously observed during iron deficiency, as Alox15 may be involved in intestinal epithelial cell differentiation and proliferation. Induction of Alox15 in the distal small and large bowels may have relevance to chronic inflammatory states, such as inflammatory bowel diseases and colon cancer, as these and other pathological conditions are known to result in anemia of chronic disease.

8.S01

Mutation analysis in French Wilson disease patients: identification of prevalent substitution and 11 novel mutations in the atp7b gene

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Wilson disease (WND), an autosomal recessive disorder of copper transport shows wide genotypic and phenotypic variability with hepatic and/or neurological and even psychiatric symptoms. The WND gene, ATP7B, encodes a copper (Cu)-transporting ATPase that is involved in the transport of Cu into plasma protein ceruloplasmin and in the excretion of Cu from hepatocytes. ATP7B mutations result in copper storage in liver and brain. Mutation analysis was carried out on 67 unrelated French WD patients (with written informed consent) presenting hepatic or neurological forms of the illness. Direct sequencing of the 21 exons and their flanking introns were performed on an AB 3130 or 3730 Genetic Analyser

equipped with seqscape software. In our WD population, 41 variants were identified, with prevalent mutations as p.M769H (exon 8), p.H1069Q (exon 14), and p.I1148T (exon 16) and 11 novel mutations, 6 missense mutations (p.A553E, p.W779C, p.K1020R, p.M1025K, p.G1287R, p.G1281D), 1 splice site mutation (c.1285–2A>G), 4 deletions (c.1738_1739delAC, c.1742_1743delA, c.3926delC, p.I1161F-fs). Most of them had strong support as disease-causing mutations, based on absence in 100 controls and pathogenic prediction on internet sites. Direct sequencing for ATP7B mutation analysis is feasible and leads to the detection of about 90% of mutated chromosomes. Molecular diagnosis of WD is very useful in clinical practice to confirm or support clinical and/or biochemical suspicion.

8.S02

Identification of ATP7A mutations associated with Menkes disease

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Menkes disease (MD) is an X-linked recessive disorder of copper metabolism characterized by progressive neurological degeneration and connective tissue abnormalities. The disease is caused by mutations in the ATP7A gene, which encodes a copper-transporting P-type ATPase. ATP7A is a large gene and consists of 23 exons spanning a genomic region of about 150 kbp. We examined mutations in the ATP7A gene in 46 unrelated patients and 1 related patient with MD. Furthermore, carrier detection was performed in 22 mothers of the patients, and prenatal/neonatal diagnosis was performed in seven male siblings. Genomic DNA was prepared from peripheral blood lymphocytes, cultured fibroblasts or amniocytes, and amplified by PCR. The direct sequencing of exons was performed with a 3700 DNA analyzer (Applied Biosystems). Forty different mutations were identified in the 43 patients; 10 nonsense mutations, 6 missense mutation, 10 splice-site mutations, and 14 insertion/deletion mutations. Most ATP7A mutations in these patients were identified in important functional/structural domains of protein products such as copper-binding domains, transmembrane domains, CPC motif, and ATP-binding domains. Eighteen of 22 mothers of patients (77.3%) were carriers of Menkes disease, while 5 mothers (22.7%) were not carriers. Four of the seven siblings (57.1%) that underwent prenatal/neonatal diagnosis suffered from Menkes disease. These results indicate that gene analysis is useful for the early diagnosis of MD and for quality of life of patients and families.

8.S03

Iron and copper toxicity in hereditary diseases

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Hereditary deposition of iron (primary hemochromatosis) or copper Wilson's disease are autosomal recessive metabolic diseases characterized by progressive liver pathology and subsequent involvement of various other organs. The prevalence of primary haemochromatosis is approximately 0.5%, about 200 times higher than the prevalence of Wilson's disease. The two diseases are characterized by homozygous occurrences of mutations in the HFE gene on chromosome 6 (primary hemochromatosis) and the ATP7B gene on chromosome 13 (Wilson's disease). Unlike most other inherited conditions, these diseases can be successfully treated, emphasizing the importance of early diagnosis. Serum ferritin values, transferrin saturation, and genetic analysis are used when diagnosing hemochromatosis. The diagnostics of Wilson's disease depends on the use of urinary copper values, serum ceruloplasmin, and liver biopsy. If untreated, both of these genetic diseases result in rapidly progressing multiorgan damage and early death. The key treatment for hemochromatosis is phlebotomy, for Wilson's disease chelation or Zn treatment. Another recently discovered disease entity (Skogholts disease) is characterized by excessive amounts of copper and iron entering the cerebrospinal fluid apparently as protein chelates, involving that

toxic amounts of the metals which may be redistributed to vulnerable binding sites on myelin, thus inducing the demyelination characterizing the latter disease. Although the present treatments of iron and copper deposition considerably improve the prognosis of patients, they may be inadequate in patients diagnosed so late that extensive body deposits of metal have been developed. The main research needs in this field are to further clarify molecular mechanisms of disease progression and to develop new chelators that are more effective and less toxic than those presently available.

8.S04

Dietary origin of simultaneous deficiency of zinc and iron in humans

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The simultaneous occurrence of zinc (Zn) and iron (Fe) deficiencies in humans is a worldwide health problem. Although the etiology is not established, circumstantial evidence supports the hypothesis that the simultaneous deficiency is derived from dietary practice. As an illustration, red meat contains both bioavailable Zn and Fe; phytate in unrefined cereals, bran, and pulses inhibits absorption of both Zn and Fe. However, it is not established that low Fe stores per se or Fe deficiency anemia conjecture low Zn status. Therefore, our objective was to identify relationships between Zn and Fe status in premenopausal women without anemia. We also examined the contribution of food frequencies and menstrual blood loss to Zn and Fe status. The subjects were 33 apparently healthy premenopausal women without anemia living in Galveston, TX, USA and nearby communities, who were not taking nutritional supplements containing Zn or Fe, or oral contraceptives. Main outcomes were Zn kinetic parameters based on the three-compartment mammillary model, using intravenous 67Zn as a tracer analyzed by inductively coupled plasma-mass spectrometry, and serum ferritin (SF) concentration; and contributing factors, the frequency of consumption of specific foods, and menorrhagia. SF was significantly and positively correlated with sizes of Zn pools. The breakpoint in the relationship between SF and the lesser peripheral Zn pool was found 21.0 μg/l of SF. SF also correlated positively with frequency of beef consumption and negatively with bleeding through menstrual pads (BTMP). Similar to SF, the Zn pool sizes correlated positively with frequency of beef consumption and negatively with BTMP. Zn pool sizes and Fe stores were highly correlated in premenopausal women. SF concentrations less than 20 μg/l suggest an increased risk of low Zn nutriture. Supported by US Department of Defense Army grant DAMD 17-95-C-5112 and accepted for publication in the British Journal of Nutrition, BJN-2006-011766R.

8.S05

Chronic copper toxicity in a nonhuman primate model: preliminary results

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Toxic effects derived from chronic exposure to excess copper are described in patients with mutated ATP7B (Wilson disease). The magnitude and length of exposure that induces liver damage, a prominent feature of chronic excess copper, is unknown in humans and nonhuman primates. We hypothesize that the first months/year of life represents a risk period to high copper exposure because at birth, liver has comparatively high copper content and biliary excretion function is immature. Therefore, increased liver amino transferases and altered liver histology will develop in nonhuman primates when exposed to excess copper since birth. We assessed clinical, biochemical, and histological effects derived from exposure to 5.5 mg Cu/kg/day in *Cebus apella*. *C.*

apella of both sexes, living in an animal house with controlled conditions received copper gluconate in their milk feedings [experimental group (GE), n=4] or a placebo with similar appearance [control group, (GC) n=4] since birth, for 2 years. A pilot study defined the copper dose to avoid acute effects. Bottles were ingested to ~100%. Clinical evaluations were performed monthly; biochemical and histological measures every 4 months during the first year and every 6 months thereafter. The IACUC (following NIH guidelines) approved the protocol. Analyses of results were conducted in a blind fashion. Animals remained clinically well and grew as expected. Preliminary data show that during the 2-year loading period, liver copper content increased in GE, serum liver aminotransferases showed no differences between GE and GC, and liver histology remained within normal limits at all times. Copper dosing failed to induce toxic effects in liver, disproving the hypothesis. Results suggest that either *C. apella* is not a good model to assess the problem or that inducing adverse effects associated with excess copper require special genetic characteristics that render individuals susceptible to copper.

8.S06

Mechanism of selenocysteine lyase: strict discrimination between selenium and sulfur

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Selenocysteine lyase (SCL) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that specifically acts on L-selenocysteine to yield L-alanine and selenium. The enzyme does not act on L-cysteine at all. We have recently found that RNAi-mediated depletion of SCL results in significant reductions in activities and protein levels of the selenoproteins glutathione peroxidase and thioredoxin reductase, suggesting an important cellular role of SCL in selenoprotein synthesis. Therefore, the strict discrimination between selenium and sulfur by SCL may be a key step in the specific selenium-delivery pathway for selenoprotein synthesis. However, the mechanism for the discrimination between selenium and sulfur by SCL remains unclear. ESI-MS analysis revealed that one selenium atom is bound to Cys-375 of SCL after cleavage of L-selenocysteine, indicating the formation of a cysteine perselenide intermediate during the catalytic reaction. Spectrum and X-ray crystal structure analyses showed that L-cysteine enters into the active site pocket but cannot form an external aldimine complex with PLP. The formation of an external aldimine complex between SCL and L-selenocysteine requires the active-site nucleophile Cys-375. A hydrogen bond, which specifically formed between the protonated thiol group of Cys-375 and the deprotonated selenol group of the substrate L-selenocysteine, probably directs the formation of an aldimine linkage between the substrate and PLP. Thus, we showed that Cys-375 of SCL plays a central role in the discrimination between selenium and sulfur in a substrate and its analog.

8.S07

The cellular function of selenocysteine lyase in selenoprotein synthesis

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Enzymatic discrimination between selenium compounds and the corresponding sulfur compounds is important for cells to metabolize selenium compounds without interference by sulfur metabolism. Mammalian selenocysteine lyase (SCL) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that specifically acts on L-selenocysteine to yield L-alanine and selenium. The physiological relevance of the selenium-specific action of the enzyme, however, has remained unclear. To address the role of SCL in mammalian cells, we have used RNA-interference (RNAi) to deplete SCL and assess its function in cell lines. We found that decreasing the level of SCL in HeLa cells results in significant reduction in protein levels of cytosolic glutathione peroxidase (cGPx) and activities of cGPx and thioredoxin reductase (TrxR). Interestingly, mRNA levels of cGPx and TrxR2 were also reduced by the SCL depletion. Depletion of SCL may cause the insufficient formation of selenophosphate and selenocysteyl-tRNASec, resulting in premature termination of selenoprotein translation at UGA codon and degradation of mRNAs for cGPx and TrxR2 via the surveillance pathway called nonsense-mediated mRNA decay. We also found that RNAi-mediated reduction of SCL induces cell growth inhibition even in the presence of selenomethionine,

selenocysteine, selenite, or FBS in a serum-free medium. One possible mechanism is that SCL may function as selenium-transferase from selenotrisulfide to selenophosphate synthetase either through a direct interaction between the enzymes or with the assistance of additional cellular components and proteins. Overexpression of mouse SCL in HeLa cells elevated the activity of cGPx, suggesting that SCL is a rate-limiting enzyme in the synthesis of selenoproteins. These results demonstrate for the first time an essential role of SCL in selenoprotein biosynthesis in mammalian cells.

8.S08

Strategy of molecular diagnosis for Wilson disease in Japan

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Wilson disease is an autosomal recessive disorder resulting from defective function of copper transport P-type ATPase (ATP7B). Copper is accumulated primarily in the liver, brain, cornea, kidney and other organs. Clinical features of this disease are liver cirrhosis, extra pyramidal signs, and Kayser–Fleischer ring. More than 200 disease-specific mutations of ATP7B gene have been reported. The mutation spectrum of ATP7B showed a population-dependent distribution. Thus, the strategy for molecular analysis and diagnosis for this disease should be established in each population. This study reports the results of mutation analysis performed on Japanese patients with Wilson disease and investigates effective strategy of molecular diagnosis for this disease. A total of unrelated 55 Japanese patients with Wilson disease were examined in this study. Gene analysis was performed under written informed consent. Genomic DNA was isolated from the peripheral blood leukocytes of the subjects. Twenty-six sets of oligonucleotide primers were prepared to amplify all exons of ATP7B gene in genomic polymerase chain reaction (PCR). The PCR-amplified DNA fragments were analyzed by direct sequencing. Gene analyses were performed under written informed consent. Twenty-one mutations were detected, including one base insertion, one or two base deletions, exon skipping, and missense mutations in the coding region. More than 80% mutations will be detected to analyze exons 8, 11, 13, and 18. These four exons should first be analyzed. We will next analyze exons 5, 10, 12, 14, 16, and 17. This is effective protocol for the molecular diagnosis for Japanese patients with Wilson disease.

8.S09

Role of zinc transporters in cadmium transport in mammalian cells

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Cadmium (Cd) is an environmental pollutant that causes adverse health effects in a variety of organisms including humans. Although the role of metallothionein (MT) in the deposition and distribution of Cd in body organs has been well investigated, the mechanism of Cd influx and efflux in mammalian cells has been poorly understood. In a previous study, we established Cd-resistant cells from mouse embryonic cells derived from MT-I, II knockout mice. The Cd-resistant MT null cells showed a marked decrease in Cd accumulation compared with parental cells primarily due to a reduced uptake of Cd. Application of multi-tracer technique revealed that the uptake of Mn is also reduced in the Cd-resistant cells. In parental cells, the uptake of Cd was inhibited by Mn and Zn, suggesting that the transport system for the uptake of Mn and Zn is involved in Cd uptake and that expression of this transport system may be suppressed in Cd-resistant MT null cells. To elucidate the differences in gene expression between Cd-resistant and parental cells, we performed DNA microarray. Among all genes that belong to solute carrier family and ATP-binding cassette transporters, expression of slc39a14 was found to be suppressed. Slc39a14 encodes a zinc transporter which belongs to a family of ZRT-IRT-like protein (ZIP). Therefore, we further determined mRNA levels of all ZIP family genes and found that expression of slc39a8 encoding ZIP8 was markedly decreased in Cd-resistant cells. To elucidate the role of ZIP8 in Cd transport, we introduced siRNA of slc39a8 into parental cells and examined cellular accumulation of metals. The cells introduced with siRNA of slc39a8 showed a decrease in ZIP8 protein level as measured by Western blotting and also a decrease in Cd accumulation. These results strongly suggest that ZIP8 plays an important role in cellular uptake of Cd and that down-regulation of

slc39a8 is the major cause for the lowered Cd accumulation in Cd-resistant MT null cells.

8.S10

Molecular regulation of the Menkes copper ATPase (Atp7a) and divalent metal transporter 1 (Dmt1) by iron in rat duodenum and IEC-6 cells

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Iron transport-related genes, including divalent metal transporter 1 (Dmt1), are induced by iron deficiency in the mammalian duodenum. We also noted very strong induction of genes related to intestinal copper homeostasis (Atp7a and metallothionein) during iron-deficiency, and intestinal and hepatic Cu loading. Intestinal copper transport may thus be enhanced by iron deprivation; in fact, our preliminary studies, utilizing a fluorescence-based transport assay, support this supposition. We thus hypothesize that Dmt1 and Atp7a are involved in increasing copper transport during iron-deficiency. Importantly, two proteins involved in intestinal iron transport, hephaestin and ceruloplasmin, are copper-dependent ferroxidases. We further show that Dmt1 protein expression is very strongly induced and present in brush-border membrane (BBM) vesicles, while Atp7a protein levels are also dramatically increased and surprisingly present on both BB and basolateral membrane domains. Additional studies demonstrate that both Dmt1 and Atp7a are induced throughout the length of the GI tract. We have now developed IEC-6 cells as an in vitro model of intestinal iron and copper transport. Dmt1 and Atp7a are strongly induced by iron chelation in pre- and post-confluent cells, and membrane associated Atp7a protein expression is also greatly enhanced. Immunocytochemical analyses show the presence of Atp7a in a cellular compartment consistent with the trans-Golgi in IEC-6 cells, with an altered staining pattern seen with iron chelation, including the presence of the protein on the plasma membrane. Additional mechanistic studies demonstrate that the induction of Dmt1 and Atp7a are likely via distinct molecular mechanisms. Overall, these studies have described novel molecular events in the intestines of iron-deficient rats and IEC-6 cells that may have relevance to understanding the mechanisms involved in enhancing iron transport during deficient states.

Category 9: Neurological diseases and neuropsychological impairment

9.P01

Effect of valproic acid treatment on serum clinical and blood hematological values in epileptic subject supplemented with selenium and antioxidant cocktails

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We evaluated the efficiency of valproic acid (VPA) treatment on different clinical and hematological values of epileptic patients supplemented with selenium and antioxidant cocktails. The levels of serum sodium (Na), potassium (K), aspartate amino-transferase (ALAT), L-creatinine, amylase, C-reactive protein (CRP), alkaline phosphatase (AFOS), hemoglobin, hematocrit, and mean cell volume (MCV) in the epileptic patients treated with VPA and supplemented with selenium (Se) and antioxidants. We also counted the amounts of erythrocytes, thrombocytes, lymphocytes, and leucocytes in the patients. The hospital staff was as the control group. All clinical measurements were made by Cobas Mira analyzer, and all hematological measurements were made by Sysmex KX-21N. Contents of serum CRP and potassium was significantly higher in the VPA group compared with the control group. Serum sodium was significantly lower in the VPA group. Content of thrombocytes was significantly lower in the VPA group.

9.P02

Effects of copper metabolism on behavioral functions and brain monoamine contents in Wistar and Wilson's disease model rats

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Alterations in copper homeostasis are implicated in the pathogenesis of certain neuronal diseases such as Menkes and Wilson's diseases. However, a few studies have examined the implication of copper homeostasis in psychiatric and neurological functions in either normal or diseased mammals. Thus, behavioral functions of Wistar and Long-Evans Cinnamon (LEC) rats, Wilson's disease animal model, were compared by measuring the open-field, acoustic startle reflex and prepulse inhibition (PPI), and shuttle-box avoidance learning tests with or without oral supplementation with copper or D-penicillamine, copper chelator. Animal handling and care were conducted according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, and all experiments were approved by the Institutional Animal Research Committee. All of the LEC rats, irrespective of the treatment, exhibited higher locomotor activity, a decreased habituation to startle response, or a lower PPI, compared with Wistar rats. The copper content of all brain regions examined, except for the medulla oblongata of LEC rats was significantly lower than those in Wistar rats. Besides, in the region of the striatum and the nucleus accumbens of the LEC rats, lower contents of norepinephrine, and higher contents of dopamine and serotonin were observed compared with Wistar rats. Although copper supplementation did not affect the brain copper content, it reduced the PPI in both Wistar and LEC rats. In contrast, D-penicillamine supplementation decreased both the brain copper content and locomotor activity, and enhanced the startle amplitude only in Wistar rats. These findings suggest that an imbalance in copper homeostasis affects monoamine metabolism and behavioral functions.

9.P03

Trace elements in serum from patients with Parkinson's disease—a prospective case-control study

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Parkinson's disease (PD) is a common, progressive neurodegenerative disorder that affects approximately 1% of adults older than 60 years. It is characterized by a progressive loss of dopaminergic neurons in the substantia nigra. The cause of the disease is still unknown, although a number of factors are likely to be involved in its etiology and pathogenesis. For example, age and dietary habits and environmental and occupational exposure to chemicals may contribute to the development of PD. We have performed a prospective study to examine whether changes in trace element concentrations could play a role in PD. Using high resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS), we have determined 44 elements in serum from PD patients and a control group. Samples were collected from patients through The Nord-Trøndelag health study (HUNT), one of the largest population-based health studies ever performed (www.hunt.ntnu.no). The serum samples used in the present study was collected in 1995–1997, and all samples were collected before the patients were diagnosed with PD. To our knowledge, this is the first study conducted on trace element concentrations in body fluids collected prediagnostically from Parkinson patients. Previous studies have indicated increased levels of various trace elements in blood from Parkinson patients, but the results have been inconsistent. In the present study, the only statistically significant difference between patients and controls was a decreased level of Hg.

9.P04

Trace elements in cerebrospinal fluid and blood from patients with a rare progressive central and peripheral demyelinating disease

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A family in Norway with a neurological disease which apparently has not been identified elsewhere

has been discovered. The disease has so far been diagnosed in three generations. It is a demyelinating disorder affecting both the central and the peripheral nervous system, and it is different from previously described hereditary demyelinating disorders. The symptom onset varies from before 30 to after 50 years of age, and the disease is uniformly gradually progressive. The exact nature of the disease, which has been named Skogholt's disease, is not yet known. We investigated whether changes in trace element concentrations could play a role in Skogholt's disease. Using high resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS), we determined 33 elements in CSF, blood plasma, and whole blood from these patients, MS patients, and a control group. Approximately threefold increased level of Cu, Fe, and Zn were found in the CSF of Skogholt patients compared to controls. Several other significant differences in trace element levels were also found. The increased levels of CSF Cu, Fe, and Zn may indicate an active role of these metals, e.g., as prooxidants, in the pathogenesis of Skogholt's disease. Alternatively, because all three metals bind strongly to proteins, and a highly increased CSF protein level is characteristic of Skogholt patients, the increased metal levels may be a secondary result of leakage of proteins into the CSF.

9.P05

Preferential dysfunction in the hippocampus in zinc deficiency

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Zinc is essential for brain function, and zinc homeostasis in the brain is strictly regulated by the brain-barrier system. Approximately 90% of the total brain zinc exists as zinc metalloproteins. The rest is histochemically reactive, exists in the presynaptic vesicles, and is stained by Timm's sulfide-silver method. Zinc-containing glutamatergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner are rich in the hippocampus. The hippocampus is responsive to dietary zinc deficiency. Learning and emotional behavior is affected by zinc deficiency. However, the involvement of hippocampal dysfunction in the change in learning and emotional behavior is poorly understood. In the present study, neurotransmitter systems in the brain were analyzed using young rats fed a zinc-deficient diet for 2 weeks. The concentrations of glutamate, GABA, and 5-hydroxytryptamine in the total brain were not different between the control and zinc-deficient rats, whereas their concentrations in the hippocampus were decreased by zinc deficiency, suggesting that the hippocampus is vulnerable to zinc deficiency. When the hippocampus was perfused by the in vivo microdialysis, the basal concentrations of extracellular glutamate, GABA, and 5-hydroxyindoleacetic acid, a serotonin metabolite, unlike that of extracellular zinc, were also decreased by zinc deficiency. In the hippocampus, glutamatergic, GABAergic, and serotonergic neurotransmitter systems are affected before zinc dyshomeostasis elicited by zinc deficiency. In the open-field test, on the other hand, the frequency of line crossing and the time of grooming were decreased after 2-week zinc deprivation. The present study indicates that abnormality of neuropsychological behavior in zinc deficiency may be linked to the preferential dysfunction in the hippocampus. The mechanism of the preferential dysfunction in the hippocampus is in progress.

9.P06

Increase in depression-like behavior of young rats during zinc deficiency

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Approximately 50% of the world population does not get adequate dietary zinc. Zinc supplementation improves neuropsychological behavior in school-age children. Zinc function seems to be linked to mental function. Dietary zinc deficiency in children is a nutritional and health problem and causes abnormal neuropsychological behavior such as lethargy (reduced activity and responsiveness). In the present study, the relationship between activation of the HPA axis and depression-like behavior was analyzed using young rats fed a zinc-deficient diet for 2 weeks. Serum corticosterone concentration was markedly increased in zinc-deficient young rats. When zinc-deficient young rats were subjected to the forced swimming test, immobility time, an index of depres-

sive behavior, was significantly increased. It is possible that the increase in depression-like behavior in zinc deficiency is linked to the increase in serum corticosterone concentration. Furthermore, corticosterone (40 mg/kg) was intraperitoneally injected into young rats once a day. Immobility time was significantly increased 7 days after the injection. To examine the effect of the increase in serum corticosterone concentration on hippocampal function, by which activation of the HPA axis is regulated negatively, hippocampal slices were prepared from zinc-deficient and corticosterone-treated rats, and the basal levels of Ca^{2+} in hippocampal cells were checked using fluo-4 FF. Intracellular calcium signals of zinc-deficient and corticosterone-treated rats were more than that of the control rats, suggesting that hippocampal function is altered by the increase in serum corticosterone. The present study suggests that sustained increase in serum corticosterone concentration not only affects hippocampal function in zinc deficiency but also increases depression-like behavior.

9.P07

Impact of dietary vitamin B12 and folic acid deficiency and anemia on school performance of Bedouin children in Southern Israel

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Deficiencies of nutrients such as vitamin B12, folic acid, and iron are frequently associated with impairment of memory, concentration, and learning ability. Deficiencies of these micronutrients are very rare in Western countries, whereas they are common in developing countries. This study was carried out to determine the impact of vitamin B12 deficiency, folic acid deficiency, and/or anemia on the academic achievement of elementary school children from a low socioeconomic population, i.e., impoverished Bedouin population living in southern Israel. Sixty-seven elementary school children, 9–11 years of age, were randomly tested. Serum levels of vitamin B12, folic acid, and hemoglobin were measured using automated chemiluminescence systems. An individual questionnaire was filled out for each student, which included information on number of meat meals consumed per week, the number of people in the family, and information about the father's employment status. Significant positive correlations were observed between number of meat meals consumed per week and low vitamin B12 levels and attainment of low marks in school, respectively. There was a negative correlation between the total number of family members and the attainment of low marks in school. No correlation between anemia or low folic acid levels and school performance was observed. Despite the small sample number, results indicated a high prevalence of vitamin B12 deficiency among these elementary school children, which could be linked to inadequate meat meal intake. This ultimately affected school performance of these children.

9.P08

The different influence of some bivalent cations on morphine-induced physical dependence

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We searched for the effect of magnesium, manganese, zinc, and copper on morphine-induced dependence in rats. Physical dependence was induced by administration of progressive escalating doses of morphine (10–90 mg/day s.c.) for 11 days at Wistar adult stars. A control group received saline and another received only morphine (M group). Other groups received $MgCl_2$ 1 mM/kg/day i.p., respectively, $ZnCl_2$ 0.1 mM i.p, $MnCl_2$ 0.1 mM/kg/day i.p, Cu acetate 0.05 mM/kg/day i.p for 11 days together with morphine in doses as in M group. Cation administration was performed 2 h before morphine administration. On the 12th day, morphine and cation administrations were stopped and were followed by a naloxone-induced (1 mg/kg s.c.) withdrawal syndrome. The other four groups received only cations in the same doses as cation+M groups and for the same period of time, and they received on the 12th day naloxone (1 mg/kg s.c.). Main symptoms of the withdrawal syndrome were surveyed for 25 min. Data obtained, statistically interpreted with ANOVA, have shown a significantly different influence of those four cations

on withdrawal symptoms. Magnesium (1 mM/kg) decreases significantly grooming, teeth chattering, aggressive posture, and erection. Manganese 0.1 mM/kg decreases significantly jumpings and locomotor activity, moderately decreased compulsive mastication but does not influence weight loss. Copper does not influence any symptoms from withdrawal syndrome. Zinc decreases grooming, "wet dog shakes" (3.17± 1.47 in the M group vs 0.83±0.75 in the M+$ZnCl_2$ group, $p<0.01$) and locomotor activity. Groups that received only cations did not exhibit any change in behavior after naloxone administration. We consider that cations interfere with acquisition phase of morphine-dependence, because changes of intensity in some withdrawal symptoms were observed after stopping the administration of cations. Variations of bivalent cations' concentrations might modulate intensity of morphine-induced physical dependence.

9.P09

Prion protein protects against zinc-mediated cytotoxicity by modifying intracellular exchangeable zinc compartmentation in cultured cells

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PrPC contains several octapeptide repeat sequences toward the N-terminus which have binding affinity for divalent metals such as copper, zinc, nickel, and manganese. However, up to now, the link between PrPC expression and zinc metabolism remains elusive. In this study, we have explored, in detail, the relationship between PrPC and zinc ions using a cell line expressing a doxycycline-inducible PrPC gene. Radioactive zinc ($65Zn^{2+}$) was used to explore the impact of PrPC expression on cellular zinc uptake. No significant difference in $65Zn^{2+}$ uptake was observed in cells expressing PrPC when compared with control cells. This result was confirmed by flow cytometry analysis, using the zinc-specific probe zinpyr-1, which shows no global difference in their intracellular exchangeable zinc content. Furthermore, spectrofluorometry quantification of total zinc corroborates the absence of PrPC expression impact on the zinc accumulation. However, PrPC-expressing cells were more resistant to zinc-induced toxicity, suggesting an adaptive mechanism induced by PrPC. Using zinquin-ethyl-ester, a specific fluorophore for vesicular free zinc, we observed a significant re-localization of intracellular exchangeable zinc in vesicles after PrPC expression. Finally, we demonstrated that PrPC expression induces metallothionein expression, a zinc-upregulated zinc-binding protein. Taken together, these results suggest that PrPC modifies the intracellular localization of zinc rather than the cellular content. Neuronal zinc dyshomeostasis appears to be a common phenomenon in several neurodegenerative disorders, and prion protein may play a crucial role in zinc homeostasis within the brain.

9.P10

Zn protects derangement of alcoholized rat brain macro- and trace elements (TE) content

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Sixty day old outbred male rats were divided in three groups: control (C), $n=10$, alcoholized (A), $n=6$, and alcoholized and treated by ZnSO4 (A+Zn), $n=7$. Ca, Mg, P, K, Na, Fe, Zn, Cu, Mn, Pb, Cd, and Sr contents of the brain cortex, hippocampus, and cerebellum, sampled after 100 days of experiment, were analyzed by ICP-OES. It was found that intragastric administration of 2 g ethanol/kg body weight daily caused significant decrease in brain cortex contents of Mg, K, Na, Fe, Cu, Zn, and Sr and increase of Pb and Cd. Gr. A+Zn demonstrated the Mg, K, Na, Fe, Cu, Zn, and Cd levels within normal range. In the hippocampus of Gr. A rats, the decreased contents of Fe, Mn were estimated. But in Gr. A+Zn, there were elevated Ca, Mg, Cu, and Zn. The Mn level in hippocampus of Gr. A+Zn was higher than in Gr. A. In contrast to cortical data, in Gr. A, the cerebellar contents of Mg, K, Na, Fe, Zn, Mn were higher, than in Gr. C. In Gr. A+Zn, the normalization of K, Na cerebellar levels were observed. So, the peroral administration of $ZnSO_4$ (0.06 mg/kg daily) has a significant protector effect on brain macro- and TE content in chronically alcoholized white outbred male rats. It prevents Mg, K, Na, Fe, Cu, Zn content

decrease and toxic metals Cd and Pb accumulation in cortex, and normalized K and Na levels in cerebellum, Mn level in hippocampus. Chronic alcoholization causes the significant redistribution of macro- and TE in the brain: decreasing of essential Mg, K, Na, Fe, Cu, Zn, Sr in cortex and increasing of Mg, K, Na, Fe, Zn and Mn in cerebellum simultaneously. Zn treatment of alcoholized rats significantly increases the hippocampal content of Zn and Cu in comparison to controls and Ca, Mg, in comparison to Gr. A and C. Possibly, hippocampus is a key structure for regulation of macro- and TE metabolism in the rat brain.

9.P11

Effect of subchronic manganese intoxication on brain morphology and manganese level in rat pups

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Long-term exposure to excessive manganese (Mn) induced clinical symptoms similar to the ones of Parkinson's disease. In the meantime, the problem of effect of the excessive Mn on the CNS of the developing organism remains obscure. We have studied morphological alterations in the neurons, distribution of the neuronal and glial cells, and calculated the glial index, as well as determined the Mn level in the n. caudatus, n. accumbens septi, n. dorsalis and ventralis Septi, and in the fronto-parietal region of the brain cortex in the 40-day-old offspring of the rats, which 15–20 days before pregnancy, during pregnancy, and until 1 month age of the litter, along with the first portion of food were given a daily of various doses (0, 10, and 20 mg/kg) of manganese chloride ($MnCl_2.4H_2O$). Intoxication induced evaluation of the Mn level in the brain of the pups and deterioration of the small part of the neurons and clear-cut gliosis. Mn exposure resulted in a statistically significant increase in cortical Mn concentration (on average, three times); Mn tended to increase in other studied brain regions. Histological analyses of brain sections indicated two morphologically distinct degenerative phenotypes of neurons: shrunk cells with indications of apoptosis and swollen cells surrounded by active astrocytes and microgliocytes with indications of necrosis. It must be mentioned, that in the cortex, the majority of damaged neurons were of the first type, in subcortical nuclei—the second type. However, the glial index was obviously increased in all studied parts of pups' brain. We consider that these shifts underlie the disorders in learning processes and emotional states, in particular, the sharp deterioration of the learning in the multi-branched maze, decrease of motor and orienting-exploratory activity, and the level of emotional tension of pups.

9.P12

Trace elements in cerebrospinal fluid of patients affected by neurodegenerative diseases: an overview of analytical data

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Metal ions homeostasis is known to be impaired in neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS). Dealing with patients affected by these diseases, accumulation or depletion of some trace elements have been documented in body tissues and fluids, sometimes with discordant results. Regarding cerebrospinal fluid (CSF), the analytical data published in the literature are really non-numerous. A few results about copper and zinc have been retrieved for each pathology; some data of aluminum are available only for AD, some values of iron and manganese for PD and of magnesium and lead for ALS. As far as other elements are concerned, the data are even lesser. Most studies have found no significant variations in the levels of the aforementioned elements in the considered diseases. However, some authors report a lower concentration of zinc in all the three pathologies, along with a higher level of copper in AD and PD and a lower level in ALS. For iron in PD, an opposite trend has been observed by two different teams. Many factors can influence the results: criteria for the enrolment of controls and patients, age, sex, severity of the disease, medical treatments, suitability and sensitivity of the analytical

techniques, statistical tests used to establish possible significances. These variables make the comparison among the literature values difficult for every matrix. Particularly in the case of CSF, the paucity of the available data and the large SDs of the mean values hamper the individuation of possible trends in trace element concentrations. Many further investigations should therefore be envisaged.

9.P13

Association of body iron stores with development among preschool children

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Iron deficiency anemia, as an extensive pandemic, has peak prevalence among young children in developing countries. Animal studies suggested a deleterious impact of iron deficiency on behavior and development markers by duration, timing, and severity of the deficiency. In humans, the evidence is inconsistent because of the constraints of small sample size and different criteria used in the definition of iron deficiency. Recent studies have shown that body iron store is a reliable indicator of iron status in populations highly susceptible to iron deficiency. We evaluated the association between body iron stores and developmental indices of children aged 1–3 years in periurban New Delhi. As part of baseline evaluation of a randomized control trial, we assessed the iron status using serum ferritin and sTfr by ELISA and development using Bayley's Scales of Infant Development II (n=709). Body iron stores were calculated using standard equations developed by Cook et al. Association of development indices with body iron store was evaluated in a multiple regression model with standardized development scores as dependent variables and body iron store, age, socioeconomic score, home score, mother's literacy, and nutritional status as independent variables. For all four models with MDI, PDI, Orientation, Emotional Regulation as dependent variables, iron stores were significantly associated with development indices after adjusting for the aforementioned covariates. MDI B 0.46 95% CI (0.21,0.72) $p<0.0001$, PDI: 0.48 (0.19,0.76) $p=0.001$, Orientation: 0.33 (−0.05,0.71 $p=0.09$, Emotional Regulation: 0.29 (0.05,0.52) $p=0.02$. MDI was independently significantly associated with age, mother's literacy and nutritional status, while PDI in addition to body iron store was significantly associated with nutritional status. This study indicates a linear association of body iron stores with both motor and cognitive development among preschool children (RCT registration NCT00255385).

9.P14

Protective effect of selenium on ciprofloxacin-induced convulsions in mice

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The quinolones are widely used antibacterial agents with broad spectrum of activity and are relatively well tolerated. However, clinical experience has indicated a possible incidence of undesirable adverse reactions including epileptogenic neurotoxicity. Although the incidence is low (~0.9 to 3.3%) and more serious CNS effects are extremely rare, seizures and hallucinations have been described more frequently in elderly and in patients with known or suspected CNS disorders. Such effects may be seen even after short-term use and are more common with high dose and advanced age and may also be seen in children. The mechanism underlying these adverse effects is still not well known. Because quinolones competitively inhibit the binding of GABA to GABA-benzodiazepine receptors, involvement of a GABAergic mechanism is thought to be essential, but the not sole component of the convulsions. The involvement of excitatory amino acid neurotransmitter, glutamate, and reactive oxygen species (ROS) has also been implicated. In fact, with rat microsomal systems, we demonstrated the generation of free radicals by the quinolone antibiotic ciprofloxacin (CPFX) by ESR spectroscopy using spin trapping technique. Our previous studies also showed that dextromethorphan, the selective antagonist of the major excitatory amino acid receptor of the brain, the *N*-methyl-D-aspartate

(NMDA) type of glutamate receptor inhibited completely the cerebral lipid peroxidation and the convulsions induced by CPFX. In this communication, we present our data demonstrating complete protection provided by selenium (1 umol/kg/day, i.g, 4 days) plus vitamin E (100 mg/kg/day, i.p, 4 days) pretreatment against CPFX-induced (257 mg/kg, i.v) convulsions and mortality in mice.

9.P15

Hepatic and presymptomatic Wilson disease patients relapsed with psychiatric symptoms due to poor medication compliance

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Wilson disease is an autosomal recessive disorder of copper metabolism. Copper accumulates primarily in the liver and subsequently in the brain, cornea, kidney, and other organs. The disease phenotype includes progressive liver cirrhosis, neurological (sometimes psychiatric) impairment, Kayser–Fleischer ring, and others. Wilson disease is a treatable disorder. Treatment involves the removal of excess copper by chelating agents, such as D-penicillamine or trienthine–2HCl, and/or blocking intestinal copper absorption with zinc salt. The treatment must be continued whole of their life. Thus, good medication compliance is very important for good prognosis. In this study, authors reported three Wilson disease patients who manifested and relapsed because of poor medication compliance. The onset ages of patients were 11, 13, and 14 years old. Two of them were diagnosed as hepatic type of Wilson disease. Another patient was found by familial analysis without any hepatic and neurological symptom. All of them were treated with copper chelating agents (D-penicillamine or trienthine), and they were getting better. However, they had stopped to take copper chelating agents for 5–10 years from 19–20 years old. As a result, psychiatric symptoms appeared in all of them. Then, two patients showed neurological symptoms. All of them lost their jobs because of these symptoms, and one patient was in a bed-ridden state because of progression of his neurological symptoms. Their relapsed symptoms were different from first ones. Thus, they delayed to recognize as relapse of Wilson disease. When the patients with Wilson disease relapsed due to poor medical compliance, a lot of them show the same symptoms with their first ones. However, different symptoms appeared in these three patients. Patients, their families and doctors have to know that Wilson disease presents variable symptoms, and good medical compliance is necessary for their good prognosis.

9.P16

Trace element fingerprints of human depression

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Depression is the most prevailing human mental condition of unknown origin in the world. The role of trace elements (TE) in depression is poorly understood. In this randomized prospective double-blind clinical epidemiology study, we analyzed the multielement profile (MP) of 41 bioelements (TE) in the human hair (H) and whole blood (WB) of 48 depressed (D) people (15 men and 33 women) and 48 apparently healthy control (C) subjects (23 men and 25 women). The major unipolar clinical depression was diagnosed by the DSM-IV criteria. Hair and WB TE MP were analyzed by the ICP MS at the CBM, Moscow, Russia. By analyzing the individual sequence pattern of TE in the hair and WB of C and D subjects, we found median (μg/g) to be the best representative for the observed data; the difference of minimum 10% of the median value was considered to be significant. A distinct TE MP pattern of emerges. Thus, for TE being higher in WB than H: H(C>D)(H<B)WB (C=D): P; H(C=D)WB(H<WB): Li; H(C<D)WB(C>D): Na, K; H(C<D)WB(C=D): Fe; H(C<D)WB(C<D): Rb. For the elements H>WB: H(C>D)WB(C>D): Se, Bi; H (C>D)WB(C=D): I, Zn; H(C>D)WB(C<D): Cu, Hg, Zr; H(C=D)WB(C=D): Cr, Co, Ni, As, Mo; H(C=D)WB (C<D): Be, Sb, Pt, Au, Tl; H(C<D)WB(C=D): Ca, Ge, Pb; and H(C<D)WB(C<D): B, Mg, Al, Si, Ti, V, Mn, Ga, Sr, Ag, Cd, Sn, Ba, La, W. Alergogenic elements Cr, Co, Ni, As, Mo were not associated with D; surprisingly, the major elements Na, K, Ca, and Mg were increased in

D. The large number of elements in category H(C<D) WB(C<D) indicates deep involvement of metals in D. The sub-analysis of the TE MP H(C>D)WB(C>D, C=D, C<D) demonstrated the essential role of iodine deficiency in the etiology of D. Depression is a complex multifactorial disease involving the dynamic interplay between the brain, metabolism, and the TE nutritional/toxicological environment.

9.P17

Zinc influence on reward system in naïve and morphine-treated rats

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We investigated the effects of Zn2+(5 and 10 mg/kg) on morphine (M)-induced conditioned place preference (CPP) on Wistar male rats. CPP is a paradigm investigating rewarding effects of stimuli, the effect being measured by noticing evolution of animals' preference over a location as a result of stimuli application. We used a CPP apparatus consisting of two main chambers different by wall aspect, linked by an intermediary chamber. Communication between chambers may be either open (CO) or closed (CC). In day 1 (preconditioning), the animal's natural preference over main chambers is determined: rat spends 15 min in the apparatus (CO). Main chamber where it spends most of the time is considered preferred chamber (PC), the other non-preferred chamber (nPC). Then (days 2 to 9—conditioning), the rat alternatively receives studied stimuli and saline (CC): in days 2, 4, 6, 8, it receives Zn2+(ZnCl2—mentioned doses), and 1 h after, M, 3 mg/kg, after which, it is placed in the nPC; in days 3, 5, 7, 9, it receives saline, after which it is place in the PC. In day 10 (post-conditioning), preference over conditioning chambers is again measured. A significant increase in time spent in nPC during post-conditioning compared to preconditioning indicates that stimulus applied in days 2, 4, 6, 8 induced CPP and has a rewarding effect. Apart from the mentioned groups (treated in nPC, days 2, 4, 6, 8 with Zn+M), there were also groups treated only with saline, M and Zn (mentioned doses). Results showed that M 3 mg/kg, induced CPP (time spent in nPC increasing from 156.4s to 547.7s, $p<0.01$). Neither dose of ZnCl2 influenced CPP model (Zn has neither rewarding, nor aversive effects). Zn, 10 mg/kg, determined a reduction in intensity of M-induced CPP (group treated only with M expressed a 250.19% increase in time spent in the nPC, while group treated with M+Zn, only 184.12% increase, $p<0.05$). This demonstrates a possible involvement of Zn2+ in morphine-rewarding effects.

9.P18

Antioxidant enzyme levels and lipid peroxidation in schizophrenic patients and their non-affected siblings

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This is a case-controlled study carried out on a group I (GI) of 60 schizophrenic patients (46 males and 14 females, mean age: 31.85±7.6 years), a group II (GII) of 33 of their non-affected siblings (18 males and 15 females, mean age: 31.63±5.4 years), and a group III (GIII) of 30 healthy controls (19 males and 11 females, mean age: 31.23±5.4 years) without family psychiatric history. The biological assessment consisted in dosage by spectrophotometer of the erythrocyte antioxidant enzymes activities: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and dosage by spectrofluorimeter of the plasma levels of thiobarbituric acid reactive substances (TBARS). The schizophrenic patients and their non-affected siblings have low erythrocyte activities of SOD and CAT compared to the control group, with respectively: 1.72 ±0.41 UI/mg Hb in GI and 1.71±0.43 UI/mg Hb in GII vs 2.46±0.32 UI/mg Hb in GIII for the SOD ($p<0.001$), and 164.05±36.88 UI/g Hb in GI, 156.88±33.45 UI/g Hb in GII vs 209.72±42.64 UI/g Hb in GIII for the CAT ($p<0.001$). The schizophrenic patients also have lower erythrocyte activity of the GSH-Px than the control group (28.32±2.22 UI/g Hb in GI vs 37.91± 2.4 UI/g Hb in GIII, $p=0.008$), whereas their non-affected siblings have higher erythrocyte activity of

the GSH-Px than the control group (47.73±2.98 UI/g Hb in GII vs 37.91±2.4 UI/g Hb in GIII, p= 0.01). The schizophrenic patients have high plasma level of TBARS. A significant difference was found mol/l μmol/l vs 1.75±0.65 μonly with non-affected siblings group: 2.15±0.9 (p=0.03). Our results showed a decrease in the antioxidant enzymes activities and an increase in the lipid peroxidation confirming the existence of an oxidative stress in the schizophrenic patients. It suggests that GHS-Px would be a protective mechanism in their non-affected siblings.

9.S01

Unique response of extracellular zinc in the ventral hippocampus against novelty stress

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An animal suddenly placed in a novel environment for a specified period shows a complex behavior for exploratory activity. This placement is a novelty stress and is regarded as painless psychological stress. An extensive neuronal activity takes place in the hippocampus during exploratory behavior. In the hippocampus, zinc serves not only as an intracellular signal factor via the interaction with many proteins but also as an extracellular signal factor in synaptic neurotransmission. However, the role of hippocampal zinc in exploratory behavior is poorly understood. To analyze the response of extracellular zinc in the hippocampus against novelty stress, rats were placed for 50 min in a novel environment once a day for 8 days. Extracellular glutamate in the hippocampus was increased during exploratory behavior on day 1, whereas extracellular zinc was decreased. The same phenomenon was observed during exploratory behavior on day 2 and extracellular zinc had returned to the basal level during exploratory behavior on day 8. To examine the significance of the decrease in extracellular zinc in exploratory activity, exploratory behavior was observed during perfusion with 1 mM CaEDTA, a membrane-impermeable zinc chelator. Locomotor activity in the novel environment was decreased by perfusion with CaEDTA. The decrease in extracellular zinc and the increase in extracellular glutamate in exploratory period were abolished by perfusion with CaEDTA. Surprisingly, perfusion with 1 μM tetrodotoxin, a blocker of sodium channels, showed the same effect as perfusion with CaEDTA. These results suggest that zinc uptake by hippocampal cells is linked to exploratory activity and is required for the activation of the glutamatergic neurotransmitter system. The zinc uptake may be involved in the response to painless psychological stress or the cognitive processes.

9.S02

Metal exposure in neurodegenerative disorders

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Neurodegenerative disorders include dementia of Alzheimer type, Parkinson disease, and amyotrophic lateral sclerosis (ALS). Disturbances in metal homeostasis or excessive metal exposure might, to some extent, contribute to neurodegenerative disorders by interfering with metalloprotein mechanisms. To estimate body burden and concentrations in tissue or body fluids of metals, lifetime exposure must be considered. Many trace elements accumulate in the nervous system, in connective tissue, or in bone. Adequate sampling includes biomarkers in tissue biopsies and in cerebrospinal fluid. The variation among individuals, the sensitivity and susceptibility in toxic effect from exposure needs attention. Epigenetic mechanisms in interplay with variations in the expression of certain metalloproteins may explain this variation. We hypothesized that metal exposure contributes to neurodegenerative disorders. We have performed detailed interviews including lifetime exposure to metals including occupational exposure in 30 patients with ALS and in controls. Occupations are classified according to standardized rating scales in relation to known metal exposure in these occupations. Occupational exposures to metals seem to be overrepresented among patients diagnosed with ALS compared to controls. Many different routes of exposure are represented. The complexity of achieving valid exposure data will be discussed. Different sampling techniques, the importance of selecting the most effective biomarkers, and the value of cerebrospinal fluid sampling will be covered.

9.S03

Involvement of zinc in synaptic plasticity and neurodegeneration in the hippocampus via cross talk with calcium

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Zinc is released with glutamate from neuron terminals in the hippocampus and serves as a neuromodulator of synaptic neurotransmission. Neural circuits of the zinc-containing glutamatergic neurons that sequester zinc in the presynaptic vesicles and release it in a calcium- and impulse-dependent manner are associated with the episodic memory function and are important for behavior and emotional expression. In the present study, the involvement of zinc in synaptic plasticity and neurodegeneration was examined using hippocampal slices double-stained with zinc and calcium indicators. Zinc suppressed the increase in calcium signal in the presynaptic (mossy fiber and Schaffer collateral) terminals induced by stimulation of depolarization, followed by suppression of exocytosis (glutamate release), suggesting that zinc serves as a negative-feedback factor of presynaptic activity. Moreover, the persistent increase in calcium signal in the CA3 pyramidal cell layer during regional delivery of 1 mM glutamate to mossy fiber synapses was significantly attenuated in the presence of 100 μM zinc, while significantly enhanced in the presence of 1 mM CaEDTA. Excitation of Schaffer collateral synapses elicited with 1 mM glutamate was also attenuated via inhibitory modulation of calcium mobilization by zinc. In excessive excitation, zinc may act protectively for CA3 and CA1 pyramidal cells initially via negative modulation of presynaptic activity and postsynaptic calcium mobilization. However, zinc taken up into pyramidal cells may damage the cells. The hippocampus is vulnerable to glutamate excitotoxicity, a final common pathway for numerous pathological processes such as Alzheimer's disease. The excitotoxicity is linked to the excessive influx of zinc and calcium. The cross talk between zinc and calcium via calcium channels such as calcium-permeable AMPA/kainate receptors may play a role in both synaptic plasticity and excitotoxicity in the hippocampus.

9.S04

Biomarkers in cerebrospinal fluid in Alzheimer's disease and normal cognition

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Major hallmarks reflecting pathological changes in Alzheimer's disease (AD) are the deposition of aggregates of β-amyloid peptides (Aβ), tau, and phosphorylated tau (p-tau) in the brain. Previous studies have indicated a relation between Aβ depositions and metals with redox activity like Cu, and Fe and with Zn, which is non-redox active but serve important roles in neurotransmission. The aim of the present pilot study was to investigate a possible relationship between the concentrations of Zn, Cu, and Fe status in cerebrospinal fluid (CSF) and relate it to proteins markers such as Aβ, tau, P-tau in CSF and cognitive function from patients diagnosed with late onset AD. The study group consisted of 32 subjects (13 females, 19 males) >63 years who were admitted to a memory clinic because of memory problems and were diagnosed with AD (case group) or with no objective memory impairment (controls SCI). Cut-off level was MMSE 28 score. CSF was sampled and analyzed for metals by ICP-SFMS. The CSF median concentration of Cu, Zn, and Fe were 13.9, 21.1, and 17.5 μg/L in the AD group which did not significantly differ from corresponding concentrations in the SCI group which were 12.4, 29.5, and 17.1, respectively. Median concentration in ng/L in AD was for Aβ, tau and p-tau 351, 819, and 89 and 670, 358, 54 in

SCIs. ApoE genotype 4:4 was seen in the AD group. No strong evidence for interactions in CSF between given protein markers and Cu, Zn, or Fe was seen. A positive correlation between Cu and Fe and tau might indicate a link between tau pathology and given metals. This study in a limited number of subjects did only to a limited extent show relationships between studied biomarkers, e.g., metals and proteins. In conclusion, the obtained results indicate that further studies on a larger number of ADs and SCIs are warranted.

9.S05

Enhancement of aggressive behavior of young mice induced with social isolation in zinc deficiency

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Dietary zinc deficiency causes anorexia, reduced gain in body weight, and growth retardation. Zinc deficiency in children is a nutritional and health problem. The stress of severe food restriction causes the increase in serum corticosterone concentration via activation of the hypothalamic–pituitary–adrenal (HPA) axis. It is possible that zinc deficiency activates the HPA axis before the decrease in zinc concentration in the brain and that this activation is linked to behavioral abnormality in zinc deficiency. In the present study, the relationship between activation of the HPA axis and neuropsychological behavior was analyzed using young mice fed a zinc-deficient diet for 2 weeks. Serum corticosterone concentration of zinc-deficient mice, in which zinc concentration in the brain was not decreased in spite of the decrease in serum zinc concentration, was markedly higher than that of the control mice. In the open-field test, the frequency of line crossing and rearing was increased by zinc deficiency, suggesting that neuropsychological behavior is altered after 2 weeks zinc deprivation. In the resident-intruder test, furthermore, isolated young mice were fed the same diet. The rate of mice that exhibited aggressive behavior to the total mice was significantly higher in isolated zinc-deficient group after 2 weeks zinc deprivation. The duration of aggressive behavior was more in isolated zinc-deficient group. These results indicate that aggressive behavior of young mice by social isolation is significantly enhanced by zinc deficiency. Serum corticosterone concentration of isolated zinc-deficient mice was also markedly higher than of the isolated control mice. On the other hand, none of zinc-deficient mice without social isolation exhibited aggressive behavior. The present study indicates that the increase in serum corticosterone concentration is not directly linked to aggressive behavior elicited with social isolation in zinc deficiency.

9.S06

Effects of a Se-restricted diet on brain antioxidant status and brain function in aged rats

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With the increasing life expectancy, cognitive decline is becoming a major public health problem. Selenium is thought to be involved in the maintenance of functional brain activity and in protection against oxidative stress. This idea is supported by the finding that the brain retains high selenium levels under conditions of persistent dietary selenium deficiency. Recently, we showed that aging was associated with a decrease in plasma selenium, while a lowered selenium status was associated with a higher risk of cognitive decline, suggesting that selenium decrease during ageing should not be underestimated as an emphasizing of brain ageing. The aim of this study was to evaluate the effects of a selenium-restricted (40 ng/g) vs normal diet (180 ng/g) on brain antioxidant status and behavioral reactivity of old rats (28 months). In rats fed selenium-restricted diet, plasma Se and GPx activity decreased significantly. The fall of Se in liver was 60% vs only 5% in cerebellum. After 12 weeks of restricted diet, we observed a decrease in complex I of the mitochondria respiratory chain assessed by Western blot, but no significant alterations in brain redox status assessed by TBARS, SH groups, GPX, GSH, and antioxidant power using ferric-reducing antioxidant power(FRAP). Furthermore, the level of DNA cortex damage assessed by comet assay was similar between

the groups. However, the Se-deficient animals exhibited more anxiety assessed by open field test. Our results suggest that Se-deficient diet during ageing could be associated with an accelerated brain ageing monitored by a decrease in mitochondrial complex I and alterations in cognitive function. In this study, these effects did not seem related to antioxidant property of Se but could be carried by a specific brain selenoprotein. Further studies are now necessary to understand by which mechanism Se acts in aging brain.

9.S07

Disturbances in acetyl-CoA metabolism and cytotoxic effects of zinc in SN56 cholinergic neuroblastoma cells

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Excessive accumulation of Zn was found in intra- and extracellular compartments of the brains affected by different cholinergic encephalopathies including Alzheimer's disease. Typical feature of these pathologies is inhibition of energy metabolism that parallels loses of cholinergic and cognitive functions in affected brains. Therefore, we investigated whether zinc may exert its cholinotoxic effects through acute and chronic interactions with principal enzymes of acetyl-CoA and energy metabolism. In homogenates of SN56 cells, Zn resulted in dose-dependent inhibition of pyruvate dehydrogenase (PDH) aconitase and ketoglutarate dehydrogenase activities (KDH) with Ki values of 0.058, 0.010, and 0.002 mM, respectively. Weak inhibition of choline acetyltransferase (ChAT) with [IC0.5] 0.3 mM was observed. Lipoamide or EDTA added to assay media before Zn abolished its inhibitory effect on each of enzymes. When added 10–15 min after Zn, they fully reversed PDH inhibition but only partially restored activities of aconitase or KDH. Chronic 24-h exposure of cholinergic cells to Zn caused concentration-dependent suppression of their proliferation and viability ([IC0.5] 0.15 mM) that correlated with depression of PDH activity ($p<0.0001$). At this concentration, Zn also suppressed aconitase, KDH, ChAT activities, cytoplasmic acetyl-CoA, and whole cell ATP by 70, 85, 33, 57, and 69%, respectively, but did not affect mitochondrial acetyl-CoA level. No changes in hexokinase, lactic dehydrogenase, succinate dehydrogenase, ATP-citrate lyase, and acetylcholinesterase activities were observed in these conditions. Lipoamide added to SN56 cells cultured with Zn fully overcame its suppressive influences on cell viability, PDH, and aconitase activities, as well as on acetyl-CoA and ATP levels and partially restored ChAT and KDH activities. Presented data indicate that suppression of acetyl-CoA metabolism may be of primary significance for cholinotoxic effects of Zn (supported by MNSW grant 2P05A11030).

Category 10: Osteoporosis and other bone disorders

10.P01

The effects of low-level cadmium exposure on bone density

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The present investigation was performed in the areas contaminated to cadmium near zinc smelter. Due to the fact that the smelter started working in 1968, the investigated population consisted of the group of people who were exposed to cadmium from their birth —former children and their parents, not environmentally exposed to cadmium at their childhood— unexposed adults. Our results obtained within the years 2000–2003 showed that environmental Cd exposure (Cd-U) in childhood might have a stronger impact on renal function, particularly tubular resorption, than the exposure of a mature organism (M. Trzcinka-Ochocka et al., Environmental Research, 95, 2004, 143–150). The present study was performed based on the group of the people investigated within 2000–2003 and consisted of 130 former children and 131 unexposed adults. Forearm bone mineral density (BMD) has been assessed by a technique—dual energy X-ray absorptiometry (DXA). The Cd-U concentrations were determined by graphite–furnace atomic absorption spectrometry (Perkin-Elmer 4100 ZL). For internal quality control, trace elements urine-

Seronorm were used. The accuracy of the method was verified via participation in the German External Quality Assessment Scheme. The obtained results indicate that environmental cadmium exposure (Cd-U) at present is lower compared to the period of 2000–2003 and decreased from 0.98 to 0.7 and 2.23 to 1.41 μg/g creatinine in groups of former children and unexposed adults, respectively. The decrease in forearm density (*T* score) is correlated with age ($p<0.002$) and Cd-U ($p<0.05$) for whole study group. The present findings indicate that the risk of low bone mineral density (osteopenia *T* score $<-1>2.5$) concerned 7% of former children and 18% unexposed adults. The symptoms of the risk of osteoporosis (*T* score<2.5) can appear only in 3% of the unexposed adult group. These results indicate that low-level cadmium exposure does not increase the risk of osteoporosis.

10.P02

Effects of dietary components on cadmium-induced bone loss in an ovariectomized rat model of osteoporosis

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Cadmium (Cd) is a heavy metal that has detrimental effects on bone. However, dietary cadmium toxicity may be affected by other dietary components. Chronic phosphorus supplementation decreases bone mass through a decline in serum calcium concentration and resultant hyperparathyroidism, but cadmium phosphate also might be precipitated in the gastrointestinal tract. Dried plum with its high concentration of antioxidants has been shown to be beneficial for bone. Our study examined the effects on Cd-induced bone damage of dietary supplementation with potassium phosphate (KPhos) and/or dried plum (DP). Fifty, 90-day-old Sprague–Dawley rats were ovariectomized and assigned to the following five treatments ($n=10$): (1) control, (2) 50 mg Cd/kg diet, (3) 50 mg Cd and 12 g potassium phosphate (KPhos)/kg diet, (4) 200 mg Cd/kg diet, and (5) 200 mg Cd and 12 g KPhos/kg diet. After 45 days of treatment, half the rats in each group had 15% DP added to their diets for an additional 3 months. At necropsy, the L4 vertebra was scanned using microcomputed tomography (uCT) to assess microarchitecture of the trabecular bone, and the femur was scanned to assess cortical thickness at the midshaft. Bone volume fraction (BV/TV) was significantly lowered by Cd and by KPhos ($p<0.0001$). Trabecular number (Ten) was significantly decreased while trabecular separation (Tb.Sp) was significantly increased by Cd and KPhos. Dried plum was beneficial in rats fed 50°Cd but not in those fed 200 ppm Cd with KPhos. Cortical thickness was decreased by Cd and by KPhos ($p<0.0001$) but increased by DP ($p=0.002$). Our results indicate that Cd causes loss of both trabecular and cortical bone. KPhos increased those losses. Dried plum increased cortical thickness; effects of DP on trabecular microarchitecture varied depending on the levels of Cd and the presence or absence of KPhos (supported by Oklahoma State University Agricultural Experiment Station).

10.P03

Open

10.P04

Open

10.P05

Does calcium supplementation during the suckling period affect bone mineral density and bone zinc later in adult rats?

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This investigation aimed at evaluating long-term effects of dietary calcium supplementation during suckling period on calcium (Ca), magnesium (Mg), and zinc (Zn) in bone. Female Wistar rats were divided in four groups (12 to 15 animals in each): (1) supplemented with 6% suspension of Ca hydrogen phosphate in cow's milk during the suckling period (Ca-supplemented) and then fed on rodent feed with 1% Ca until the age of 3 months; (2) Ca-supplemented and then fed on 0.45%

Ca; (3) sham-supplemented with cow's milk as suckling (sham-supplemented) and then fed on 1% Ca; (4) sham-supplemented and then fed on 0.45% Ca. At the age of 3 months (young adult rats), whole body bone mineral density (BMD) was measured by dual energy X-ray absorptiometry, and Ca, Zn, and Mg concentrations were analyzed in femoral bone tissue by atomic absorption spectrometry. The experiment was conducted in accordance with the NIH Guidelines for the care and use of animals and approved by authorized national institutions and Institute's Ethical Committee. Two-way analysis of variance evaluated the effects of Ca supplementation during the suckling period and the effects of two levels of Ca intake (1% or 0.45%) during the weaning and after weaning on BMD and bone Ca, Mg, and Zn. The results showed that Ca supplementation during the suckling period did not affect whole body BMD and bone Mg concentration in young adult rats, Ca concentration in the femur decreased 6.6%, and bone Zn concentration increased 5%. Animals fed on 1% Ca in feed had a slightly higher whole body BMD (2%), higher Ca concentration in the femur (9%), and lower bone Zn concentration (7%) than rats fed on 0.45% Ca. Bone Mg was unaffected. To conclude, under these experimental conditions Ca supplementation during the suckling period has no beneficial effect on bone mineral density in the adulthood. Effects on bone Zn deserve further exploration.

10.P06

The feasible use of teeth as the site for the in vivo quantification of strontium by X-ray fluorescence spectrometry

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Strontium (Sr) has gained much attention over the last few decades because of its chemical/biochemical similarity to calcium (Ca). Like Ca, Sr is a bone-seeking element and has been shown to be responsible for various bone disorders, particularly rickets and osteomalacia, in areas of high dietary Sr consumption. People living in areas subject to nuclear fallout have been shown to have increased incidences of various cancers due to elevated consumption of foods high in Sr-90. This study demonstrates that the tooth, including baby teeth, can act as the site for the painless, nondestructive in vivo or ex vivo quantification of Sr by energy dispersive X-ray fluorescence spectrometry (EDXRF). The Sr signal induced by an I-125 source (in the form of brachytherapy seeds) was successfully normalized to the Ca signal from the enamel. The relative SD of the Ca signal for all teeth was 5.7%, indicating that the Ca content in enamel is reasonably constant regardless of the individual's age, sex or race (n=42, p<0.05). Enamel Sr was quantified from the normalized Sr signal in conjunction with a calibration curve produced from a series of hydroxyapatite enamel phantoms purified of Sr (2 ppm). Validation of the EDXRF-determined Sr concentrations was performed by comparison with graphite furnace atomic absorption spectrometry, which indicated that the EDXRF method can be used successfully as a means of quantifying dental Sr with a minimum detectable limit of 28 ppm. Dental Sr concentrations from this study ranged from 50 to 300 ppm, with a 95% confidence interval of (169±80) ppm, and the results appeared to be clustered. Such large variation is not often observed and may be a reflection of the population diversity in Toronto (Canada), an immigration hub, from which the sample was drawn. Likewise, the clustering may also be an effect of the variety of ethnic communities and traditional diets represented in the sample.

10.P07

Trace element concentrations in human nails using atomic absorption spectrometry

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Evaluation of trace elements in human tissues such as hair and nails proves useful in the studies pertaining to chronic body exposure. These may have been also considered as indexes to evaluate environmental exposure. Nail samples were collected from healthy

people aged 5–60 years. Samples were digested with nitric and perchloric acid; the elements were then determined by atomic absorption spectrometry. These elements are calcium, iron, magnesium, and zinc. Results show that calcium has the highest concentration compared to other elements. Elemental concentrations are compared to literature values, and chronological variation is illustrated. A pattern of the concentration of elements in nail samples were analyzed regarding age and sex.

10.P08

Elimination of arthritis pain and inflammation for over 2 years with a single 90-min, topical 14% gallium nitrate treatment: case reports and review of actions of gallium(III)

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Gallium(III) can inhibit the production of inflammatory cytokines, such as IL-1beta, produced by macrophage-like cells in vitro. A dose-dependent inhibition of IL-1beta and TPA stimulated MMP activity by gallium nitrate at increasing concentrations occurs, demonstrating that gallium nitrate can be a useful modulator of inflammation in arthritis. Gallium (III) is an inhibitor of bone resorption and is an effective treatment for hypercalcemia. Gallium(III) has been reported to be effective in the treatment of *Mycobacterium butycicum*-induced arthritis in rats by antagonism of iron III. Long-term elimination of pain from arthritis by gallium(III) was first observed in horses primarily being treated for navicular disease. Several people treating their horses with gallium nitrate coincidentally found that arthritis pain in their fingers ended and did not return after soaking their hands in 14% gallium nitrate solution. Therefore, the severely arthritic hands of a 60-year-old woman were topically treated with a 14% aqueous solution of gallium nitrate for 90 min. Pain and inflammation from rheumatoid arthritis diminished rapidly, and neither pain nor inflammation returned during the following 2 years from that single treatment. A 61-year-old woman who had osteoarthritis in her left knee, shoulders, and wrists was treated orally with 50 ml of a 1% gallium nitrate solution (120 mg elemental gallium) daily using a 2-week on and two week off protocol, resulting in almost total elimination of pain while on gallium nitrate, while pain partially returned during the 2-week off periods. Treatment of frozen shoulder with topical 40% gallium nitrate for 120 min resulted in greatly reduced pain and crepitus almost immediately with complete restoration of range of motion, with pain remaining essentially absent for over 1 year. Proper use of gallium(III) may be effective in terminating pain and inflammation of arthritis for years, often with a single treatment.

10.P09

The assessment of the effect of calcium, magnesium, and vit D supplementation by the hair multielement profile follow-up

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The aim of this study was to show the changes of the hair multielement profile (MP) after calcium, magnesium, and vit D supplementation. The study was conducted by following the principles of Helsinki Declaration and approved by the IMI Ethical Committee. The hair trace element (TE) MP was analyzed in three perimenopausal women who participated as control subjects in the randomized double-blind prospective clinical epidemiology study of human depression. Calcium, Mg, and Si content of the hair was analyzed by the ICP MS at the CBM, Moscow, Russia. All three women were supplemented according to their individual bioelement status. Women A, B, and C have increased Ca, Mg, and Si (A), increased Ca and Mg (B), and normal Ca, Mg, and Si (C), respectively; they were supplemented accordingly to the individual MP TE. All women received vit D. TE deficiencies were corrected at the intermittent intervals. Simultaneous supplementation of Ca, Mg, and Si in Subject A resulted in the decrease of hair Ca, Mg, and Si. Simultaneous supplementation of Ca and Mg in Subject B resulted in the decrease of Ca and Mg; whereas Si which was not supplemented, has increased. The Subject C showed an increase of all the three elements in the hair, in percentiles before

and after supplementation: A [Ca: 3.6, 2.0; Mg: 1.9, 1.4; Si: 3.3, 2.2], B [Ca: 2.1, 1.0; Mg: 2.6, 1.4; Si: −0.2, 0.2], C [Ca: −1.2, 1.5; Mg: −0.4, 2.4; Si: 0.8, 1.3]. Simultaneous nutritional supplementation with Ca, Mg, and vit D is associated with the decrease of their concentration in the perimenopausal women's hair, what we named "hair calcium paradox". We suggest that many negative reports on suitability of the hair analysis for the assessment of the Ca nutritional status are biased by not realizing the existence of the hair calcium paradox, i.e., the counterintuitive fact that more Ca and Mg in the hair means less Ca and Mg to the bone and visa versa.

10.S01

Recovery of bone status after abandoning ingesting cadmium-rice for 10 years

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Cadmium may have both direct and indirect effects on bone metabolism. The main focus of this study was to evaluate whether stopping ingestion of cadmium-polluted rice is associated with the bone recovery status. In this study, a total of 459 persons (296 females and 163 males) participated. The residents in control area ingested rice with a cadmium concentration of about 0.072 mg/kg all the time; those living in the moderately and highly exposure area stopped ingesting the cadmium-polluted rice since 1996. The participants completed a questionnaire, and the bone mineral density was measured by pDEXA at the radius and ulna. The change in forearm bone density between 1998 and 2006 was used as markers of recovery status. The results showed that the bone density declined linearly with age ($p<0.05$), and those of the residents living in highly exposed area were significantly lower than those of residents living in the control area in females at the age of 50 and 70 years old ($p<0.05$), as was the same in males at the age of 70 years old. The values of decline of groups of UCd are 5–10 μg/g creatinine and above 10 μg/g creatinine, and Bcd above 10 μg/L was significantly higher than those of low exposure groups (in females $p<0.05$, in males $p>0.05$). The decline values and percentage changes in BMD were positively correlated with the cadmium (in females $p<0.01$, in males $p>0.05$). It is concluded that bone recovery is not obvious, and the negative effect of cadmium on bone still remains, even the population has abandoned ingestion of cadmium-rice for 10 years, especially in females.

10.S02

Se deficiency and manifestation of Urov disease in East Siberia

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Urov Kashin-Beck disease is a heavy osteoarticulate disease of humans. The disease etiology is not precisely determined until now. In the People's Republic of China, the disease emergence has been associated with selenium deficiency in the environment. However, the selenium-deficient territories of China cover some more extensive areas compared to the areas of the Urov disease endemic location. Therefore, the purpose of this work was to reveal the location of selenium-deficient territories of the Chita region in view of the human and animal endemic diseases spread. Selenium and its different forms were determined through a spectrofluorimetric method. It was discovered that the biospheric taxons examined are characterized by the significant deficiency in selenium connected to some geological and/or climatic features. The selenium deficiency found most frequently in the areas were granitoids, and inundated meadow-alluvial soil types are spread. On the soils containing carbonate undergrounds (like limestone or marble) and those locally spread within the East Zabaikalia boundaries, the selenium mobility rates are sufficiently higher and such sites are free from endemic manifestations. Nevertheless, selenium deficiency is typical not only for the Urov endemic spread territories. Low rates of selenium biogenic migration are specific not only for the endemic villages in the Chita region, but also for other territories. The analysis of Se mobile forms in the soils of East Zabaikalia and East Meshchera shows that distribution of some separate forms in the soils of

the Urov disease—unaffected villages and some villages—near Moscow are practically similar.

10.S03

The Ba/Sr ratio, carious lesions, and dental calculus among the population buried in La Concepción (Tenerife, Canary Islands)

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Some trace elements may accumulate in bone in relation to dietary habits, so some of them are of interest in paleodietary analysis. This is the case of Sr and Ba, wherein potential value as paleodietary indicators is enhanced by their relatively inert metabolic behavior. On the other hand, the presence of carious lesions may indicate consumption of vegetables, whereas etiology of dental calculus deposition is multifactorial, although some authors consider them as indicators of protein consumption. Based on these factors, we analyzed in the present study (1) Ba, Sr, and calcium contents of trabecular bone obtained from the ramus mandibularis, by atomic absorption spectrophotometry; (2) presence of teeth with carious lesions; 3) presence of teeth with carious deposition in 37 mandibles from the church La Concepción, an 18th century burial place from Tenerife. Sex was assessed genetically, by analysing genetic expression of amelogenin. We found that (1) carious lesions were observed in 18 cases, and dental calculus, in 19; no association was observed between sex and presence of dental caries or calculus deposition, nor with the proportion of carious teeth nor the proportion of teeth with carious lesions; (2) people buried near the altar (priests and individuals of the highest social class) showed a significantly higher proportion of teeth with dental calculus deposition; (3) bone Sr was moderately (346.6±64.5 ppm), but significantly, higher than the values observed in a control group composed by 20 modern omnivorous individuals; (4) the Ba/Sr ratio was significantly higher among individuals with carious lesions and kept a direct correlation with the proportion of carious teeth. These data suggest that there were differences in dietary pattern among the individuals belonging to different social classes; overall, consumption of vegetables was widespread, as shown by the high proportion of carious lesions, in relation to raised Ba/Sr ratio.

10.S04

Knee osteoarthritis and its correlation with daily boron exposure

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This study aimed to determine knee osteoarthritis (OA) and its relation to high B exposure and estimation of daily B exposure in the same population. The study group consisted of 66 males living and working in an area using water containing at least 2 mg/L of B. Ten were excluded from the study with the history of past knee problems. The controls (*n*: 56) never worked or lived in a B-rich area. ACR criteria were used for OA diagnosis. For estimation of daily B exposure in a study group formed from the region, there were 583 males older than 20; 66 males with the required qualifications were studied, 57 served as controls from elsewhere. The estimation of daily boron exposure was based on 24-h urine analysis by ICP-OES method. One way Annova was used for calculations MINITAB. Median age was 44.5 (38 to 84), height 170 cm (157–185), weight 80 (61–105 kg) with a median body mass index (BMI) of 27 (22–36) in study group. The control's median age was 48 (37 to 70), height 170.5 cm (160–185 cm), weight 77 kg (60–115), and BMI 26.5 (20 to 37). The presence of knee osteoarthritis in the B-exposed group was14/56 (25%), and in the controls, this was 25/56 (44.6%; Chi-Square tests were significant). In the study group, average daily B exposure level was 6.768±0.473 mg/day with an average weight of 73.98±1.64 kg. In controls, these values averaged 1.256±0.104 mg/day and 74.60±1.56 kg. Daily exposed B level per

kilogram of body weight was 0.093±0.006 mg and 0.017±0.001 mg, respectively. It can be concluded that the study group had been exposed to boron by a factor larger than 5. There were no reported health problems of subjects in group I, and no accumulations of death due to a certain disease which may be linked to high B exposure. These values may be considered as safe levels. Although genetic factors cannot be eliminated—only known difference is exposure to B levels-OA was significantly less in studies. Therefore, if the diet is supplemented with B, the symptomatic OA may be prevented.

Category 11: Toxicity of trace elements

11.P01

The cellular effects induced by Pb^{2+} and Zn^{2+} action on human erythrocytes and lymphocytes

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Depending on doses, TE can exert two kinds of effects on functioning in the organism, being either essential constituents of biological molecules of various importances or toxic factors to bring serious diseases. The latest data were given using literature and proper information. A number of experiments to elucidate the membrane mechanisms of action of low lead concentrations on human erythrocytes (HEC) were performed. Using fluorescence probes and EPR labels, lead was shown to change the microviscosity of lipids in isolated (HEC) membranes at low (2–6 mM) concentrations, intact donor HEC, and children HEC with lead content higher than maximum permissible level. Similar data were obtained for lymphocytes with help of fluorescence probes but at higher (ten and/or 50 mM) lead concentrations. Lead-induced lipid changes were accompanied by the structural changes in band 3 protein and NADH-methemoglobin reductase both in HEC in vitro and erythrocyte of children with lead content in blood being higher than a MPL. Zn^{2+} is essential for several hundreds proteins, for DNA stabilization, gene expression, etc., but under high concentrations, it induces several intracellular effects accompanied by severe dysfunctions that are shortly considered here with particular reference to the properties of zinc as an antioxidant. Zn^{2+}-induces the structural changes in HEC membranes that follows from the presented data. Besides, atom force microscopy allowed imaging of the topography of membrane surface with a great deal of particles having several nanometers in size. Based on the above data, the structural state of blood cell membranes was concluded to be able to serve as a criterion in severity of intoxication.

11.P02

Zinc concentration in human autopsy and body fluids

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Zinc is involved in many biochemical processes that support life. It is a constituent of various tissues and essential for more than 200 enzymes, etc. However, zinc deficiency or excess intake or disturbances in its metabolism lead to disease and toxicity. Data on the zinc concentration in the human body are important, for example, to estimate the amounts occurring in healthy persons and found in cases of chronic and acute poisonings with zinc compounds. In this paper, the concentration of zinc in human tissues and body fluids obtained from autopsy cases of non-poisoned people (n=193), aged from 14 to 80 years, between 1990 and 2007, is presented. The following values were found by flame atomic absorption method (mean±SD, median, range, in μg/g or μg/ml): brain 10.6±2.14, 10.3, 6.03–15.8 (n=50); stomach 13.2±4.07, 12.7, 5.18–22.5 (n=72); intestines 14.3±4.60, 15.8, 8.15–28.1 (n=30); liver 37.1±17.3, 35.2, 11.0–78.8 (n=108); kidney 32.5±10.7, 31.0, 10.8–60.9 (n=90); lung 11.2±3.90, 10.1, 4.92–18.7 (n=27); spleen 14.7±2.53, 14.6, 11.4–18.3 (n=5); heart 26.5±3.63, 26.7, 22.5–31.8 (n=5); blood 8.13±2.11, 7.89, 4.02–12.8 (n=71); bile 4.92±1.64, 4.20, 3.20–7.09 (n=9). The accuracy of the method was checked through the use of standard reference material, Bovine Liver, 1577b (certified: 127±16 μg Zn/g, found: 117±0.7 μg Zn/g (n=6)).

11.P03

Estimation of common effect of *Eleutherococcus senticosus* (rupr. et maxim. ex maxim) extract and cadmium on mitotic and apoptotic activity of liver cells

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An area uninvestigated for immunomodulators such as *Eleutherococcus senticosus* (ES) is the interaction between these compounds and heavy metals. It is estimated that biologically active compounds from plants can have a protective action during intoxication by heavy metals. The aim of the study was to evaluate the effect of ES extract on the accumulation of cadmium in liver, mitotic and apoptotic activity of liver cells after the chronic intoxication by Cd2+. Experiments were carried out on the white laboratory mice. Mice (n=57) were periodically i.p. injected for 6 weeks with CdCl2 (0.05 LD_{50} Cd^{2+}) and ES extract solutions of two different concentrations (0.05 LD_{50} and 0.1 LD_{50}) and their combinations. The control group of mice was injected with 0.9% saline. Cadmium concentration in spleen specimens was determined by atomic absorption spectrophotometer Perkin-Elmer/Zeeman 3030. The number of mitotic liver cells was counted in ten randomly selected reference areas (0.04 mm2). Apoptosis of liver cells was histochemically detected by the TUNEL assay using AP (Roche) in situ cell death detection kit. Preparation of extract from roots of ES was made in the factory "Valentis" (Lithuania). Cd^{2+}concentration in liver of mice in group ES (0.05)+Cd was −1.61-fold higher, therefore in group ES (0.1)+Cd was1.43-fold higher comparing to cadmium group. The mitotic activity of liver cells induced by Cd^{2+}after injection of ES extract was the same as in control group. ES extract did not induce apoptosis of liver cells, but decrease apoptotic activity induced by cadmium. Long-term injections of extract of ES (0.1 LD_{50} and 0.05 LD_{50}) combined with $CdCl_2$ (0.05 LD_{50}) leads to the significant increase of cadmium concentration in liver of experimental mice. ES decreases the mitotic activity and apoptosis of liver cells induced by cadmium.

11.P04

Comparison of effects of *Echinacea purpurea* and *Eleutherococcus senticosus* (rupr. et maxim. ex maxim) extracts on the accumulation of cadmium in different organs "…Mitotic activity of liver cells"

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Cadmium disturbs the activity of biochemical systems of cells. *Echinacea purpurea* (L.) Moench (EP) and *Eleutherococcus senticosus* (Rupr.et Maxim.ex Maxim) (ES) can modify it's influence. The aim of the study was to compare the effect of EP and ES extract on the accumulation of cadmium in blood, liver, and kidney and mitotic and apoptotic activity of liver cells after the chronic intoxication by Cd^{2+}. Experiments were carried out on the white laboratory mice. Mice (n=100) were periodically i.p. injected for 6 weeks with $CdCl_2$ (0.05 LD50 Cd^{2+} and EP, ES extract solutions of two different concentrations (0.05 LD50 and 0.1 LD50), and their combinations. The control group of mice were injected with 0.9% saline.Cd^{2+}concentration in blood and organs was determined by AAS Perkin-Elmer/Zeeman 3030. The number of mitotic liver cells was counted in ten randomly selected reference areas (0.04 mm2). Apoptosis of liver cells was histochemically detected by the TUNEL assay using AP in situ cell death detection kit. Cd^{2+} concentration in liver of

mice in group EP(0.05)+Cd was1.78-fold higher, therefore, in group EP(0.1)+Cd was 2.11-fold higher compared to the Cd group, in the kidneys 2.1-fold and 1.92-fold respectively, and in the liver of mice in group ES (0.05 LD50)+Cd, it was1.61-fold higher, therefore in group ES (0.1 LD50)+Cd was 1.43-fold higher compared to Cd group in kidney 1.72-fold and 1.90-fold, respectively. EP and ES decreases the mitotic activity of liver cells induced by cadmium. EP arises apoptotic index (AI) of liver cells comparing with control group, but ES did not induce AI. Long-term injections of extracts of EP and ES (0.1 LD50 and 0.05 LD50) combined with $CdCl_2$ (0.05 LD50) and leads to the significant increase of cadmium concentration in blood and organs of experimental mice. EP and ES decrease the mitotic activity of liver cells induced by cadmium. EP increases the apoptotic activity of the liver cells, but ES decreases this factor.

11.P05

Toxic methylation of dimethyltin to trimethyltin in human

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Dimethyltin dichloride (DMT) is widely used for a heat stabilizer in polyvinyl chloride. DMT is of low toxicity, but trimethyltin chloride (TMT) is highly toxic to the central nervous system. Occasional TMT poisoning occurred from careless use in a worldwide scope. We reported a case of acute DMT poisoning with neurologic manifestation very similar to TMT encephalopathy. Then, we examined whether the methylation of DMT to TMT occurred in humans using speciation analysis of methyltins in the urine of the patient with acute DMT poisoning. Monomethyltin trichloride (MMT), DMT, and TMT in urine were analyzed using combination of high performance liquid chromatography (HPLC, Model HP-1100, Agilent, USA) and inductively coupled plasma-mass spectrometry (ICP-MS, Model HP4500, Agilent, USA), using m/z=118 and 120 of tin. MMT, DMT, and TMT were separated using a cation-exchange column (RSpak NN-614, Shodex, Japan) and a polymer column (ODP50, Shodex, Japan). To confirm the chemical species of the detected peaks, mass-spectral analysis was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Quatromicro API, Waters, USA). Chromatographic peaks in the patient' urine were identical with the standard solution made by dissolution of DMT and TMT reagents. Although biotransformation of alkyltin has been reported to be demethylation in mammals, the DMT taken in by inhalation was found to be methylated to TMT in a human body in our case. Both DMT and TMT were detected in urine even 40 days after the acute exposure and the concentration of TMT (57.7 μg Sn/L) was much higher than that of DMT (27.4 μg Sn/L). Our study clarified toxic methylation of DMT to TMT in human and the conversion rate was high. It is required to strictly control DMT use.

11.P06

Strategies for safe and effective therapeutic measures against metals: role of micronutrients

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The heightened concern for reduction of environmental pollution that has been occurring over the past 20–5 years has stimulated active continuing research and literature on the toxicology of metals. Man has succeeded in poisoning himself with metal toxins repeatedly throughout recorded history. We can take steps to understand this threat and put into action policies of prevention and treatment that may help to lessen the negative impact that these agents have on human health. Once these chemicals are inside us, we can never fully eliminate them unless we undergo a detoxification process. As a result, most of us need some form of detoxification. The present investigation reviews the acute, subchronic, and chronic exposure of mercury, vanadium, and beryllium in rats. The effect of exogenous applications of glutathione, D-penicillamine, NAC, and DTT were studied in combination with Zn, Se, Mg, and Fe in the acceleration of metal elimination. Activity of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, triglycerides, cholesterol, and HDL were significantly elevated with the administration of toxicants. A rise was found in hepatic, renal, and neuronal lipid peroxidation with a concomitant decline in reduced glutathione ($P<0.05$). The enzymatic activities of GSH cycle, such as GPx,

GR and G-6PDH were also altered due to oxidative damage. A fall was observed in the enzymatic activity of AChE in fore-, mid-, and hindbrain. In the present study, Zn, Se, Mg, and Fe, along with chelating agents, were effective in mobilizing all the metals from the soft and hard tissues, preventing against deleterious metal-mediated free radical attacks, thus, protecting biological membranes from damage. The reversal of intoxication has been discussed.

11.P07

Gene expression analysis of the mouse brain perinatally exposed to methylmercury and/or polychlorinated biphenyls

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Methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are environmental pollutants that cause neurobehavioral deficits in human. As the exposure levels to both compounds are low, it is necessary to consider the various confounding factors. Fish is the main source of human MeHg exposure and PCBs exposure. In this experimental study, we investigated the effect of combined perinatal exposure to MeHg and PCBs in terms of neurobehavioral development and gene expression analysis (microarray one-color method) of the offspring. Female mice (C57BL/6Cr) were exposed to MeHg (5 ppm as Hg in diet), PCBs (Aroclor 1254, 18 mg/kg/3-days), PCBs + MeHg, or vehicles. Before weaning of offspring, developmental observations were made. At 8 weeks old, they were tested by an open field test and water maze test. Several developmental and neurobehavioral tests showed significant effects of MeHg alone or PCBs alone. Effects of interaction of treatment with PCBs + MeHg were also observed in the open field test. The results of microarray analyses (whole brain on postnatal day 1) showed increased number of genes with alternated expression. The number of genes that were commonly expressed in MeHg group and PCBs group was small. MeHg + PCBs group expressed larger number of genes that were not expressed in MeHg group and PCB group. In conclusion, the gene expression pattern by co-exposure was different from those by exposure to MeHg alone or PCBs alone. It is of interest to correlate the results of behavioral tests and the gene expression in future study.

11.P08

Effect of nickel on the testicular and epididymal structure and its distribution in the rat organism after an experimental administration

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Nickel compounds are known human carcinogens and are also known for their dermatologic effects. In the study, the effects of nickel on the structure of the testis and epididymis and the concentration of the metal in the rat tissues were studied. Nickel chloride was administered intraperitoneally to adult male Wistar rats at dose level 25 (group A) and 35 mg/kg (group B) body weight. Another group (P) of 4-week-old males was given 100 mg/L in drinking water for 3 months. The changes in the testis and epididymis were evaluated 48 h after an i.p. administration and after 3 months of peroral exposure using the morphometry methods. The nickel concentrations in the kidney, liver, testis, and muscle tissues were analyzed using the electrothermal atomic absorption spectrophotometry. The morphometry analysis of the testes revealed a significant ($p<0.05$) increase in the interstitium relative volume and the decrease in the tubule lumen volume in group A. The absolute epithelium volume significantly ($p<0.05$) decreased in group A. In the epididymis, a significant increase in the relative volume of the epithelium ($p<0.001$) and absolute volume of the epithelium ($p<0.001$) and interstitium ($p<0.01$), the decrease in the lumen relative and absolute volume ($p<0.001$) were observed at the highest dose group (*P*). The absolute weight of epididymis significantly ($p<0.05$) decreased in group

A. The histopathological changes in both organs were observed. The nickel concentrations in the kidney increased significantly in group A and P ($p<0.05$ and $p<0.001$, respectively). A significant ($p<0.05$) increase in the Ni amount in the testes was also noted. On the contrary, the Ni amount in the muscle tissue significantly decreased in group B and P ($p<0.01$ and $p<0.05$, respectively). The results indicate that both i.p and oral exposure to nickel affects the testis and epididymis histology and increases the amount of metal in the kidney and testes (supported by VEGA grants 1/2417/05 and 1/3475/06).

11.P09

Speciation of mercury in the tissues of the rats which received dental amalgam fillings

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Methylation of the mercury released from dental amalgam placed in the oral cavity has not been established. Recently, Leistevuo et al. (2001) reported that the amount of organic and inorganic mercury in paraffin-stimulated saliva was significantly higher in subjects with dental amalgam fillings than that in the nonamalgam study groups. They also demonstrated that the results were compatible with the hypothesis that amalgam fillings may be a continuous source of organic mercury. We, therefore, carried out this study on the possibility of methylation of mercury released from dental amalgam fillings, examining the amounts of total mercury (T-Hg) and methylmercury (MeHg) in the tissues of rats as to their relationship to amalgam fillings. Thirty-six rats were given one, two, or four maxillary molar amalgam fillings and bred for a full 6 months. Another 12 rats, which received no fillings were used as controls. Total mercury was measured by reducing vaporization-gold amalgamation atomic absorption spectroscopy, while methylmercury was measured by ECD-gas chromatography. Amounts of total mercury in brain, liver, kidneys, and blood samples were found to be higher in all rats receiving fillings than in the controls. Although amounts of methylmercury in liver and kidneys from the experimental animals were higher than that of the controls, amounts of methylmercury in blood samples were almost the same as in the controls. We could not conclude that the possibility of methylation of mercury is released from dental amalgam fillings from this study.

11.P10

Health consequences of exposure to diet-sourced environmental cadmium in Central Jamaica

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There are concerns in Jamaica regarding the incidence of chronic diseases such as diabetes, hypertension, osteoporosis, and renal disease. Some trace elements such as lead (Pb) and cadmium (Cd) are known nephrotoxins. Cd in particular, damages the proximal tubules of the kidney and has been linked to the occurrence of renal disease. Proximal tubular dysfunction results in decreased reabsorption of certain compounds including glucose, amino acids, and calcium into the bloodstream by the kidneys, resulting in excretion to the urine instead. Reduced absorption of these compounds may cause renal disease, glucosuria, proteinuria, and osteoporosis, similar to Fanconi's syndrome. Soil surveys carried out by ICENS show elevated concentrations of Cd in the mainly bauxitic soils of Central Jamaica. The highest concentrations are 100 to 1,000 times higher than levels typically found naturally worldwide. Some crops for local consumption and export are cultivated on these Cd-rich soils, and elevated concentrations of Cd have been found in tubers and green leafy vegetables. Autopsy studies of kidney Cd concentrations confirm elevated human exposure, with results showing that persons from central Jamaica exceed the general population average by a factor of two. Diet studies have ascertained that a population in Central Jamaica is at risk of being exposed to Cd levels in excess of the PTWI set by the WHO. Cd exposure and proximal tubular dysfunction were measured as urine Cd (U-Cd) and B2 microglobulin (B2-M) in urine, respectively. Elevated levels of U-Cd and B2-M concentrations were confirmed with a strong correlation between the soil Cd and the U-Cd. Higher B2-M

concentrations (>200 μg/g creatinine) were found in the population with U-Cd concentrations greater than 2.5 μg/L. This raises a concern that the impairment in the reabsorption capacity of the renal tubules may be contributing to various chronic conditions.

11.P11

Selenium as an antioxidant in the thyroid gland

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The purpose of the study was to investigate the relationship between selenium status as an antioxidant and the thyroid gland function. The thyroid contains more selenium per gram of tissue than any organ, and selenium like iodine, is essential for appropriate thyroid hormone synthesis, activation, and metabolism. The data suggest that selenium is mainly present in the follicular or thyrocyte compartments. This would appear reasonable because thyroid follicles continuously produce H_2O_2 for thyroid hormone synthesis throughout life. The cytotoxic effect of H_2O_2 on thyroid cells induced caspase3-dependent apoptosis that occurs at H_2O_2 concentrations that are sufficient to induce necrosis. Therefore, the thyrocyte need a very potent antioxidative defense system against H_2O_2 and reactive oxygen intermediates derived thereof. We performed a descriptive, observational, cross-sectional study in the setting of an endocrinology clinic. To evaluate the thyroid function, TSH, thyroxine, T4, T3, T3RU, and FTI levels were measured in the serum of patients. Two arbitrary serum-TSH threshold levels (TSH<1.0 and>4.0 mU/L) were introduced to classify, respectively, hyperthyroidism and hypothyroidism, and euthyroid conditions (1.0<TSH<4.0 mU/L), and each patient was assigned to one of these groups. Serum selenium levels were determined by graphite furnace atomic absorption spectroscopy with nickel as a matrix modifier for background correction (AA670 G-SHIMADZU). Data analysis was performed by SPSS NO-13 software. The differences among serum selenium in euthyroids, hypothyroids, and hyperthyroids were statistically significant (p<0.005), and the serumic selenium levels in hyperthyroids patients were lower than other groups. Our finding suggests that selenium as an antioxidant trace element protects against thyroid dysfunction.

11.P12

Morphofunction of the condition of the liver upon toxic organism loading of doses of cadmium and lead in diets with mineral substances

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The researches directed on revealing features of a structurally functional condition of a liver at in a diet of toxic and essential elements on of Wistar rats. In a period of 21 days, four groups (n=20) of animals were fed with a diet containing minimal contents of iodine, selenium, and zinc (control group). To individuals of group I, a diet containing lead sulfate (0.004 mg/head/day) and cadmium sulfate (3 mg/head/day) was administered. In the diet of group II, toxic elements in the form of potassium iodide (0.332 mg/head/day), Se-lenopyran (0.0001 g/head/day), and zinc sulfate (0.042 mg/head/day) were introduced in the diet. In the diet of group III, essential elements were introduced in the same dosages. Upon examination of the organisms given Cd and Pb, it was found out that in the hepatocytes, glycogen is absent; in separate segments, it is observed that there is vacuolization and necrosis of the center lobules in the hepatocytes. Places significant zones in the sizes necrosis. In the center lobule there is a 5.7 increase in the quantity of bicyclic cells (p<0,05); in the control group, the increase is 1.35%. In a hepatic fabric of the group II animals, the absence of glycogen in cells is one of its characteristics. In hepatocytes wherein glycogen is not absent, these are found to be abundant in vacuoles. This phenomenon is considered as a display of mechanism adaptation. Upon introduction in a diet of a complex of microelements I, Se, Zn, the majority of the hepatocytes in the cytoplasm is enriched with glycogen. The expressed difference between the center lobules and the peripheral cells is not observed. In

comparison with the control, the number of cells in the hepatocytes, with average and greater kernels, increases in volume.

11.P13

Distribution and metabolism of tellurium in rats ingested with sodium tellurite

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Tellurium (Te) is one of the important metalloids used in industries and is ubiquitously found in the environment. However, the biological and toxicological effects of Te are still unclear despite its being recognized as a hazardous element. Recently, Te, as an alloy with germanium (Ge) and antimony (Sb), is found to be of use in digital versatile disk-random access memory (DVD-RAM) and DVD-rerecordable disc (DVD-RW). This study was performed to gain an insight into Te metabolism in the body on the basis of speciation analysis of metallomes. The mechanism for the discrimination of Te from selenium (Se), an essential metalloid belonging to the same group as Te, was also clarified. Rats were given drinking water containing tellurite and 82Se-labeled selenite at the same concentration, and the concentrations of these metalloids in organs, body fluid and excreta were determined 2 days later [All animal experiments were performed according to the "Guide for the Care and Use of Laboratory Animals" (issued by NIH) and the Guidelines of Institutional Animal Care and Use Committee, Graduate School of Pharmaceutical Sciences, Chiba University, Japan, and have been approved by the committee]. The results demonstrate that urinary and fecal excretion of Te was, respectively, lower and higher than that of exogenous (labeled) Se, suggesting that Te was less absorbed than Se. Incorporated Te bound to selenoprotein P and globulins but not to albumin in serum after Te was taken up by RBCs, and the Te bound to these plasma proteins was transported to organs. The transported Te was transformed, i.e., methylated in organs and effluxed into bloodstream, and the effluxed Te was highly accumulated in rat RBCs in the form of dimethylated Te. In contrast, Se was not accumulated in RBCs. Finally, Te was excreted in urine as trimethyltelluronium. The results suggest that the metabolism of Te was distinct from that of Se in rats.

11.P14

Assessment of effect of *Eleutherococcus senticosus* (rupr. et maxim. ex maxim) extract on the accumulation of cadmium in spleen and the extent of macrofagus area

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Cadmium has a diversity of toxic effects including nephrotoxity, carcinogenicity, teratogenicity, and endocrine and reproductive toxicity. There is the evidence of therapeutic benefits of *Eleutherococcus senticosus* (ES). The aim of the study was to evaluate the effect of *E. senticosus* extract on the accumulation of cadmium in spleen and the extent of the macrofagus area after the chronic intoxication by Cd^{2+}. Experiments were carried out on the white laboratory mice. Mice (n=57) were periodically i.p. injected for 6 weeks with $CdCl_2$ (0.05 LD50 Cd^{2+}) and *E. senticosus* extract solutions of two different concentrations (0.05 LD50 and 0.1 LD50) and their combinations. The control group of mice were injected with 0.9% saline. Cd^{2+}concentration in spleen specimens was determined by atomic absorption spectrophotometer Perkin-Elmer/Zeeman 3030. The extent of macrofagus area was estimated using the immunohistological reaction by antibody CD68. Preparation of extract from roots of *E. senticosus* was made in the factory "Valentis" (Lithuania). Cd^{2+} concentration in spleen of mice in group ES (0.05)+Cd was 3.378 μg/g; therefore, in group ES (0.1)+Cd, it was 3.133 μg/g and in Cd group, it was 1,904 μg/g. The extent of macrofagus area was less in groups: ES (0.05)+Cd and ES (0.1)+Cd compared with Cd group. Long-term injections of extract of *E. senticosus* (0.1 LD50 and 0.05 LD50) combined with $CdCl_2$ (0.05 LD50) leads to the significant increase of cadmium

concentration in spleen of experimental mice. *E. senticosus* decreases the activity of macrofagus induced by cadmium.

11.P15

A Wilson disease patient with recurred acute hepatitis due to poor medical compliance

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Wilson disease is an autosomal recessive disorder of copper metabolism. Copper accumulates in the liver, brain, cornea, kidney, and other organs. The disease phenotype includes liver dysfunction, neurological impairment, Kayser-Fleischer ring and others. Patients with liver dysfunction may present with acute hepatitis, recurrent hepatitis picture, cirrhosis, and/or liver failure. Wilson disease is a treatable disorder. In this paper, the author reported a case of Wilson disease with recurred acute hepatitis because of very poor medical compliance. The case is an 18-year-old female. When she was 10 years old, she had symptoms of general fatigue and abdominal pain. Her AST and ALT levels were elevated. Her laboratory findings also revealed a low serum ceruloplasmin level and high urinary copper excretion. She was diagnosed as having Wilson disease, and treatment with D-penicillamine was started. Her liver dysfunction had been getting better. However, her medical compliance was not so good, and when she was 13 years old, she stopped to take D-penicillamine completely. Until 18 years old, no hepatic symptom was presented. When she was 18 years and 2 months old, she presented with fever. Then she felt general fatigue and abdominal pain. Physical examination revealed icterus and Kayser–Fleischer rings. Serum AST and ALT levels were highly elevated. We diagnosed her as having acute hepatitis of Wilson disease due to poor medical compliance and some viral upper respiratory infection. We restarted D-penicillamine from a dose of 800 mg/day then up to 1,400 mg/day. Her general fatigue and abdominal pain disappeared in a few days, and AST, ALT, and total bilirubin levels fell to normal levels in about 1 month. Wilson disease patients have to continue therapy for their whole life. Poor medical compliance will cause miserable prognosis. Education for patients and their family to keep good medical compliance is very important for the care of Wilson disease.

11.P16

Effect of trace elements in a complex preparate (Immunovet-HBM™ trade name) on the activity of digestive enzymes in broiler chickens

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The effect of feed supplemented with Immunovet-HBM™ (contained microelement complex: Zn, Mn, Fe, and Se) has been tested by altogether 36, 1 day old ROSS 308 chickens. The chicken stock was devised into two groups. The control group consisted of 18 (3×6), the experimental group of 18 (3×6) chickens. The Immunovet-HBM™ supplementation was given to the feedstuffs of the experimental group, namely, to the growing and finishing diets of animals. The weekly measured live weight gain of chickens consistently was higher in the experimental group, and the end of trial final body weight of Immunovet-HBM™ supplemented fed broilers (2,152 g) was 7.06% higher than in the control group (2,010 g). The feed conversion efficiency was 1.72 kg/kg (9.00% $p<$ 0.01) in the treated group, which is less than in the control group, 1.89 kg/kg. In this paper, the effect of the prebiotic in feedstuffs was studied on the activity of hydrolase's enzymes in the broilers' pancreas tissue and intestinal content. The observations were made on broilers of 0 day which were kept in climatized animal facilities. During the trials, six groups were created. The animal number in each groups was 36 (control group's 3×6=18, Immunovet-HBM™ 1 g/feed/kg treated group 3×6=18 broiler chickens). The animals were euthanized on day 42 on the control and treated group. The measurement of digestive enzyme activity was made on the pancreas tissue and intestinal content of 36 animals from each group. The data justify that the supplying of growing and finishing diets of broilers by supplementation could improve the fattening parameters, and the environmental pollution could also be reduced. The digestive enzyme activity was significantly different between the experimental and the control groups. According to the results, Immunovet-HBM™ can significantly modify

the hydrolase's activity both in the pancreas and in intestinal content.

11.P17

Ameliorative effects of *N*-acetyl cysteine along with the combination of antioxidants against chronic exposure induced by methylmercury toxicity

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The mutual antagonism of mercury and thiol compounds is one of the strongest and most gen0065ral example of interaction in the trace element field. Mercury is a systematic poison which causes neuro-, nephron, and hepatotoxicity. Present investigation was carried out to study the ameliorative effects of chelating agent (NAC) with and without nutritional supplements Zn and Se. NAC is a natural sulfur-containing amino acid and consists of sulfhydryl group for binding electrophilic species. Zn and Se are universal antioxidants, which prevent free radical generation, protecting biological membranes from damage. It recycles the body's enzymatic and nonenzymatic defence systems. Adult male rats of Sprague–Dawley strain weighing 150+10 g were divided into six groups of five animals each. Group 1 served as normal control. Groups 2–6 were administered dimethylmercury (1 mg/kg, p.o.) for 12 weeks (5 days/week), and group 2 served as experimental control. Group 3 was treated with the therapy of NAC (2 mM/kg, i.p.). Animals of groups 4, 5, and 6 were treated with NAC + Zn (2 mM/kg, p.o), NAC + Se (0.5 mg/kg, p.o) and NAC + Zn + Se, respectively, for 2 days after toxicant administration. A significant rise was observed in the AST, ALT, ALP, gamma glutamyl transpeptidase, albumin, bilirubin, urea, and creatinine, which indicated the leakage of enzymes from the liver and the kidney resulting in alteration in the cell permeability. A sharp elevation was observed in LPO; however, reduced GSH, ATPase, G-6-Pase, and glucose-6-phosphatase dehydrogenase activities were also decreased in the liver, the kidney, and the brain, indicating oxidative damage resulting in the free radical generation. An appreciable fall was observed in the activity of acetyl cholinesterase in different regions of the brain. *N*-acetyl cysteine, along with nutritional supplements, was found to be effective; however, NAC + Zn + Se was the most effective combination therapy. This is also supported by light and electron microscopical study.

11.P18

Effectiveness of combination therapy against subchronic exposure of aluminium

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Aluminum is present in air, water and food and has been considered an indifferent element from a toxicological point of view and may be mediated, at least in part, by free radical generation. The chronic use of medicines and fruit juices containing citrates greatly increase absorption of Al. A causal role in dialysis encephalopathy, microcytic anemia, and in Alzheimer's disease is well known. It decreases spontaneous nervous discharge, thereby, reducing nervous activity. Combination therapy is a new novel strategy. The present experiment was carried out to determine the effect of combined therapy of NAC and HEDTA along with Ca and Fe. Group 1: Normal control (normal saline 4 ml /kg i.p.), group 2: experimental control (1/50 LD50 of Al (NO3)3 i.p. for 21 days + 5 days rest), group 3: Al (NO3) 3 i.p. for 21 days followed by NAC + Ca + Fe for 5 days, group 4: Al (NO3) 3 i.p. for 21 days followed by HEDTA + Ca + Fe for 5 days. Activities of AST, ALT, TG, cholesterol and LDL were significantly elevated, and HDL was inhibited after toxicant administration. The rats exposed to Al showed significant increase in the BUN, creatinine, and urea level disrupting the kidney function. The activity of AChE was inhibited in all the parts of the brain. In addition, LPO and GSH levels were also altered in the liver, the kidney, and the brain during toxicity. The activities of GPx, GR, and G6PDH were decreased in the liver and the kidney, whereas marginal inhibition was noted in the brain's GPx and G6PDH when compared with control groups. Combination therapy showed over all improvement in all the biochemical changes. Al exposure increased collagen fibers and degenerated cristae of mitochondria in the liver. Well-formed mitochondria, endo-

plasmic reticulum, and nucleus were seen after therapy of HEDTA + Ca + Fe. Histopathological changes in the liver, the kidney, and the brain were also recouped with the therapy. Distribution studies also supported the results. In conclusion, HEDTA was more effective when compared to NAC.

11.P19

Investigation of cytotoxic effects of thallium acetate and ellagic acid on NIH 3T3 cell line

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In this study, we searched out the cytotoxic effects of thallium, a toxic trace element affecting the nervous system, and ellagic acid (a natural polyphenolic compound which is widely found in fruits and nuts) on cells for an NIH3T3 lineage. To establish the most effective results, we applied nine different concentrations of thallium and ellagic acid (1, 10, 25, 50, 100, 250, 500, 750, 1000 μM). In this study, cytotoxic effects were evaluated using MTT method after 24-h incubation of the NIH3T3 cell line. To determine the effects of these compounds on lysosomal activity, we dyed the cells with neutral red after a 24-h incubation in thallium and ellagic acid with concentrations of 1, 10, 100, 250, 500, 750, 900, 1,000 μM. Incubation of NIH 3T3 with ellagic acid at a concentration of 500 μM dose resulted in 56% cell viability. Thallium incubation with NIH 3T3 cell cultures resulted at 500 μM 78.7% after 48 h. There is a decrease in the cell viability, at a concentration of 500 μM 39.5% after 72 h. Ellagic acid had a more cytotoxic effect than thallium on the NIH3T3 cell line.

11.P20

Speciation analysis by HPLC-ICP-MS of arsenic in urine of individuals drinking polluted water in Bangladesh

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The toxicity and carcinogenicity of arsenic depend on its species. Individuals living in Bangladesh drink water polluted with high levels of inorganic arsenic species. Speciation analysis of urinary arsenic is required to clarify the health risks of arsenic intake. There has been no report of urinary arsenic analysis in Bangladesh using high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). A community-based cross-sectional study was performed in the Pabna district of Bangladesh and involved 332 adult participants, including 165 men (42.7±10.9 years) and 167 women (34.9±9.6 years), who were directly interviewed and submitted urine samples and samples of drinking water from 138 wells between May and July 2005. We performed speciation analysis of urinary arsenic using HPLC-ICP-MS. The median concentration of arsenic in the 138-well water samples was 37 μg/L (interquartile range: 4–86 μg/L). The median values of urinary arsenic species were as follows: arsenite (AsIII), 17.4; arsenate (AsV), 2.1; monomethylarsonic acid (MMA), 14.2; dimethylarsinic acid (DMA), 89.3; arsenobetaine (AsBe), N.D.; arsenocholine (AsCh), N.D.; trimethylarsine oxide (TMAO), N.D.; unidentified arsenics (others), 2.5; and total arsenic (total As), 127.0 ugAs/l. The median creatinine-adjusted values were as follows: AsIII, 45.0; AsV, 4.4; MMA, 35.8; DMA, 234.6; AsBe, N.D.; AsCh, N.D.; TMAO, N.D.; others, 3.5; and total As, 114.9 ug As/g creatinine. Our findings indicate that high levels of AsIII, AsV, MMA, and DMA in urine were due to exposure to high levels of inorganic arsenic species in drinking water. In addition, our findings indicate that absence of AsBe, AsCh, and TMAO were due to lack of ingestion of organic arsenic species from seafood.

11.P21

Role of metallothioneines in the mechanism of heavy metal toxic effects

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The role of metal transporting proteins (MTP) in an organism is an answer for linkage and transport of essential and toxic metals. Despite intensive study of these proteins, many aspects of heavy metal transport

remain unexplored. Therefore, the purpose of the present research was determining, in an acute and subchronic experiment with white rats, the role of MTP in the mechanism of toxic action of inorganic Cd and Hg after intragastric exposure in doses at a level Limac and Limchr. The phase character of metal linkage was fixed: nonspecific, specific linkage, and disintegration of metal–protein complexes. There are differences of these process rate and specific capacities for different MTP, as it is shown on albumin and metalothioneins (MT) examples. Entered into an organism, heavy metal at the first minutes of exposure practically completely binds with albumin and other organic ligands. The inductive synthesis of MTs is responsible for the second stage of metal linkage. A high correlation rate ($r>0.78$–0.85, $p<0.05$) between the contents Cd and Hg in the liver and kidneys exposed animals with the particular contents of MT in blood and urine is determined. Also, the role of MT in cellular transport and subcellular distribution of heavy metals has been shown. MT has a signaling role in lysosomal enzyme activation. The last are responsible for the destiny of metal–MT complexes and their influence on inclusion and expressiveness of adaptive mobilization of the cellular protective mechanisms. One of the main mechanisms is the adjustable level of the oxidative stress, defined on lipid peroxidation and antioxidant parity; first of all, SOD, glutathionperoxidase and other antioxidant enzymes activity. Their imbalance forms, finally, a cellular syndrome of metalotoxicosis.

11.P22

Small doses of mercury exposure: what does it mean?

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During the last years, the increasing attention of research is involved in a problem of "micromercurialism", which is connected with the influence of small doses of mercury. A system of Hg transport, detoxication, and removing from the organism is carried out at the cellular level. So, the purpose of the given research was studying the action of small doses of Hg and determining their hygienic importance. In the subchronic experiments on male white rats, the action of $HgCl_2$ in different doses (1/200, 1/1,000, 1/10,000 and 1/100,000 of DL50) on different markers of cellular metabolism was examined. It was shown that independent on a dose ions of mercury in bioenvironments are found as complexes and adductors with proteins, while free Hg^{2+}in the examined biosystems were practically absent. In allocated by differential centrifugation subcellular fractions of the liver, kidneys and brain cells, the growth of Hg accumulation in the brain cells mitochondria—in 10, liver and kidneys—almost in 1,000, and in lysosomal fractions of kidneys—near 2,000 times in relation to the control is revealed. The mercury content in brain homogenate has grown in 2.7, in kidneys—in 26.2, in the liver—in 30.3 times. There were marked dose-dependent distinctions in the changes of nuclear, mitochondrial, lysosomal, and cytosol enzyme activity, the intensity of lipid peroxidation, contents of SH-groups, and other investigated biomarkers of exposure. Materials of the carried out research allow attributing to category "small" such dozes of Hg, which are at the Limchr level and lower and are determined with the help of system of the interconnected chemical and biological markers. The last can be used for the hygienic control, risk assessment, and carrying out of preventive, medical, and rehabilitation measures.

11.P23

Hygiene and toxicology of Zn- and Pb-containing ship paints

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Epidemiological examination of 874 ship painters has shown a 34% of the surveyed workers with functional disorders, which can be classified in the category of professionally caused. It is a cause of harmful working conditions, factors of labor process and, also, presence of corresponding properties defined in air of a working zone of chemical substances. For persons with experience of more than 10 years, these symptoms were seen four times more often than in the non-experienced

group of painters. According to the character of functional changes in an organism, syndromes of general toxicity (central and peripheral neuropathy—39.6%; respiratory disorders—34.7%; vasocardial syndrome—25.8%; hepato- renal syndrome—17.4%) and dermatotoxicity (local dermal—58.3%; the general with dermal component—17.2%; allergenic—9.9%; mixed or dermato-allergic—14.6%) were determined. Studying the role of heavy metals in development of the revealed disorders and their pathogenic mechanisms, a series of experiments on white rats with lead and zinc acetate in doses of 50 mg/kg and, also, dermal application of Zn- and Pb-containing paints has been placed. The maintenance of lead, zinc, calcium in blood, urine, liver, kidneys, and brain tissues was determined with atomic-absorption method and the wide spectrum of biochemical markers. There are established signs of tissue hypoxia, activation of free-radical oxidation processes, inhibition of antioxidant defense systems of biomembranes disorders, enzymopathy, that will be correlated with data of clinical research and can be used for early diagnosis and treatment of functional infringements at ship house painters.

11.P24

New aspects of metallonephrotoxicoses pathogenesis

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Mechanisms induced by heavy metal (HM) renal pathology development have represented an indefatigable interest. It is determined by the role of HM as global pollutants and their complex, diverse character of their interaction with the hepato-renal system. The diagnosis of metallonephrotoxicosis is put only at the acute or chronic renal insufficiency formation. Quite often, the etiological factor remains unknown. Therefore, the purpose of the given research was experimental, studying features of the initial stages of what were caused by HM nephropathy for, their more effective early diagnostics and treatment. White mail rats within 4 weeks daily were injected intraperitoneally HM solutions of $CdCl_2$ and $HgCl_2$ in doses of 0.1 and 1.0 mg/kg, Pb (NO3) 2 in dozes of 1.0 and 10 mg/kg. Animals were on standard hyposalt-containing diet. Weekly water and hypersalt (0.9% water solution NaCl) loading were carried out for an estimation of renal functional reserves. Diuresis, osmolality, protein, creatinin, K+ and Na+ levels in urine were determined. Also, the glomerular filtration speed (GFS), K+ and Na+ reabsorption and clearance, and osmotically active substances were calculated. The carried out researches have allowed to establish generally all investigated HM and characteristic attributes of metalotoxicosis. Discrepancy between defeat proximal tubules has been revealed in the action of Cd and Hg, and change of size diuresis, proteinuria, infringement Na reabsorption and GFS. Reduction of GFS depends on the tubule damage more the renal blood-groove decrease. It has been confirmed at the direct renal blood-groove definition in rabbits after introduction of $HgCl_2$. The new metallonephrotoxicoses classification and system of informative biomarkers for their diagnostics are proposed.

11.P25

Cadmium in urine and kidney biomarkers in Swedish women

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The general population is exposed to cadmium via diet and smoking. The uptake is higher among women with low iron stores. Signs of renal effects of cadmium have recently been demonstrated also at relatively low exposure levels. The aim of the present study was to assess cadmium exposure and possible renal effects in a population sample of women in the Western part of Sweden. This study was part of an environmental health monitoring programme. One group included 104 middle-aged women (50–59 years, mean 55) recruited at their visit to a mammography unit. The other group was recruited by mail and included 85 young women (20–29 years, mean 24). The participation rate was 89 and 70%, respectively. Information about smoking habits, number of children, and dietary habits was collected by a questionnaire. The Ethics Committee approved the studies. A first morning urine sample was collected, and cadmium was analyzed by ICP-MS. Quality control

samples were analyzed in the same runs as samples from the study. NAG and creatinine were also determined in the same samples. Out of the middle-aged women, 27% had UCd above 0.5 μg/gC vs only 3.5% of the young women. The median levels in the two groups were 0.37 μg/g C and 0.14 μg/gC, respectively, and for active smokers, the levels were 0.57 and 0.17 μg/gC. In the middle-aged group, a significant correlation was found between NAG and UCd in nonsmokers. It is important to reduce the cadmium body burden. This can be obtained by reducing smoking in the young population. In the middle-aged group, 10 years of smoking increased UCd with about 0.1 μg/g creatinine. Cd in the diet should also be reduced by decreasing emissions and deposition in soil. Earlier studies have shown a more marked effect at lower UCd levels in the general population than in workers exposed to cadmium by inhalation. One may speculate that cadmium intake by food might affect the kidneys more than inhalation of cadmium.

11.P26

Cesium toxicity arising from anticancer therapy

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Cesium is an alkali metal with no known biological role in human health. CsCl is marketed as an alternative therapy for oncology patients but has been implicated in clinical toxicity syndromes. We present a case of a 45-year-old woman who was treated with CsCl for breast cancer. After 4 months of therapy, she developed recurrent syncopal episodes. On admission (day 0), cardiac monitoring revealed a QTc prolongation that culminated in ventricular fibrillation (torsades des pointes). She was defibrillated, resuscitated, and has a normal electrocardiogram reading after lidocaine followed by mexilitine therapy. Serum, whole blood, and urine samples were collected. 133Cs levels were measured by ICPMS using a Perkin Elmer ELAN 6100 equipped with a dynamic reaction cell, dual detector, Meinhard type quartz nebulizer, quartz cyclonic spray chamber, platinum skimmer, and sampler cones, and AS90 Plus autosampler. Samples were diluted in an alkaline Triton X-100/EDTA/gold/*n*-butanol detergent using 193Ir as an internal standard. Cs replicate values were interpolated from a calibration curve generated using serial dilution of a single element Cs standard. Ir was used as the internal standard. The analysis showed serum Cs levels of 48, 45, and 35 mg/L, on days 0, 11, and 29, respectively. Whole blood Cs results were: 246, 180, 120 mg/L on days 1, 11, and 29, respectively (>100 X values in controls). A 24-h urine collection (day 11) had a Cs level of 262 mg/L (62000X value in controls). She had concurrent hypokalemia (3.0 mmol/L) on day 0. Several animal and human studies have reported CsCl-induced cardiac arrhythmias. The toxigenic mechanism appears to be Cs-induced blockade of potassium channels leading to prolonged repolarization of cardiac myocytes. This impairment in repolarization leads to generation of a Q on T phenomenon that predisposes individuals to ventricular fibrillation. While CsCl toxicity is well documented, its anticancer properties remain only speculative.

11.P27

Maternal-to-fetal cadmium transfer and its effects on trace element regulation in the reproductive organs of female rats

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Maternal-to-fetal transfer of Cd and the effect on trace element (zinc, copper) regulation was investigated in the reproductive organ and newborn rats. Mother rats were given Cd orally (1, 2, 5 mgCd/kg) for 6 days a week. Cd administration was continued during the mating, the gravid, and the lactation periods. Concentrations of Cd, Zn, Cu, metallothionine (MT) were measured in the liver and kidney of neonate rats, and the immunohistopathological examination of uterine

and placental tissues was performed on the 19th day of pregnancy. Cd accumulation increased dose-dependently in uterine and placental tissues of the mother rat depending on the increase of Cd intake. Cu concentration in placental tissue decreased corresponding to Cd accumulation. Remarkable decrease of Cu accumulation in high molecular weight and MT fraction, corresponding to the increased accumulation of Cd in both the fractions of placental cytosol, was found at the 19th day of pregnancy. The induction of MT protein in placental tissue was saturated to the Cd accumulation in the groups of 2 and 5 mg Cd/kg. On the other hand, the atrophy disorder of syncytial trophoblast of placental tissue was found depending on the increase of Cd intake in groups receiving 2 or 5 mg Cd/kg, and particularly prominent in the 5 mg Cd/kg group. Cd concentration in liver and kidney of neonate rat (1 day old) increased depending on the increase of Cd intake of mother rats. Cd concentration level in the liver of neonate rats was higher than that of kidney. Zn and Cu concentrations in liver and kidney of neonate rats decreased with corresponding Cd accumulation of both organs. From the results of Cd distribution of neonate rats, it was suggested that Cd is not bound by MT and was passed or leaked into the fetus through the uterine–placental barrier by damage to the syncytial trophoblast.

11.P28

Tetrathiomolybdate for Wilson's disease inhibits copper-dependent lipid peroxidation

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Wilson's Disease (WD) is a rare autosomal recessive genetic disorder which causes copper to be elevated in the blood, urine, liver, and brain. Current therapies using copper chelation (e.g., penicilliamine) are shown to cause neurologic worsening in neurologically presenting WD. Of concern is that these therapies increase the availability of unchaperoned circulating free copper which, by its very chemical nature, may cause generation of toxic reactive oxygen species. In fact, studies show that in WD, lipid peroxidation (LP) and its by-products are elevated giving rise to additional secondary pathologies including cancer. We are developing tetrathiomolybdate (TM) as a drug for the treatment of WD. The mechanism of action of TM involves a unique complexation and neutralization of free copper in the stomach, intestines, and blood stream. As such, we wanted to know if TM would inhibit copper-dependent LP. To test this hypothesis, we developed a model of LP using linoleic acid micelles. Linoleic acid micelles (10 mM) were incubated with copper sulfate or buffer for 18 h at 40°C. We then made visible-absorbing adducts of malonyldialdehyde and hydroxyl nonenal (by-products of LP) by reaction with TBA and MBTH, respectively. LP of linoleic acid micelles after incubation was then quantified by absorbance readings at 414 nm. The results indicated that copper-dependent LP was dose and time dependent. Based on these findings, linoleic acid micelles were incubated with copper sulfate at 100 μM for 18 h at 40°C, as conditions for testing drugs that inhibited LP. These conditions gave absorbances that were eight times above background. TM inhibited LP by 40 and 90% at 10 and 100 μM, respectively. By comparison, Trientine inhibited LP by only 17% at 1,000 μM and penicillamine actually increased LP by 30% at 1,000 μM. The concentrations of drugs tested in the experiments described above represent clinically relevant doses. Mechanism of action will be provided.

11.S01

Immunolocalization of metallothioneins in liver and kidney of Wistar rats exposed to cadmium

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The present study aimed to investigate the induction of metallothionein (MT) in rats exposed to cadmium chloride ($CdCl_2$) via drinking water. Thirty-two Wistar rats, including both genders, were given

cadmium chloride in drinking water at the doses of 0, 2.5, 5.0, 10.0 mg Cd/day kg body weight, respectively, for up to 12 weeks. After killing, paraffin-embedded liver and kidney tissue blocks were sectioned and subjected to immunohistochemistry for MT localization. The significant immunoreactive MT (irMT) was observed in tissues of rats at the highest dose, which showed a dose–effect relationship. Furthermore, rats in each group had stronger MT staining in the kidney that was localized mainly in renal cortex and less irMT expression in renal medulla, compared to the liver. It has been demonstrated that cadmium exposure results in significant MT synthesis, but the quantity of expression varies with and within organs.

11.S02

Role of selenium in seafood risk: benefit evaluations

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Maternal consumption of common varieties of ocean fish has not been associated with adverse fetal neurodevelopmental outcomes. Instead, recent studies find that increasing maternal fish consumption benefited child neurodevelopment, while maternal avoidance of fish had adverse consequences. The protective effects of selenium (Se) against Hg toxicity have been recognized for over 50 years and have been demonstrated in all animal models evaluated. As interactions between Se and Hg and their molar ratios in seafood are essential factors in evaluating risks associated with dietary Hg exposure, considering Hg content alone is an inadequate means of assessing risk. In this study, the absolute and molar concentrations of Hg and Se were determined in edible portions from 420 individual fish representing 15 species of pelagic fish collected from the central North Pacific Ocean near Hawaii. A molar excess of Se in relation to Hg was observed in most fish species. The rank order of Se:Hg molar ratios was striped marlin (17.6)>yellowfin tuna (14.1)>mahi-mahi (13.1)>skipjack tuna (12.8)>spearfish (11.4)>wahoo (10.8)>sickle pomfret (6.7)>albacore tuna (5.3)>bigeye tuna (5.2)>blue marlin (4.1)>escolar (2.4)>opah (2.3)>thresher shark (1.5)>swordfish (1.2)>mako shark (0.5). Mako shark may be hazardous for maternal consumption, as its Se:Hg ratio approaches that of pilot whale meat (~0.25), an uncommon food known to be associated with adverse effects on child development. Although ocean fish are generally Se-rich, the amount of protective Se present in fresh water fish can vary dramatically. Consumption of fish with the same Hg concentration that are not associated with harm in a Se-rich fish could cause significant harm if present in a Se-poor food. Comprehensive criteria that incorporate the absolute and relative amounts of Se and Hg present in fish are needed to improve dietary Hg risk assessment and assure quality maternal nutrition for optimal child development.

11.S03

Zinc protoporphyrin is a strong predictor of lead levels in developing country settings where lead levels are high

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Chronic lead poisoning and iron deficiency are highly prevalent in urban children. Studies have shown a link between iron deficiency and elevated blood lead levels. Lead initiates ferrochelatase inhibition, which impedes binding of iron to protoporhyrin IX, leading to protoporphyrin accumulation. This increase in protoporphyrins serves as a lead exposure marker. This tool was discarded in developed countries because of a decline in lead levels below the linear association range. In developing countries with high prevalence of lead, it can still be a useful tool. As a part of randomized controlled trial, we evaluated the association of zinc protoporphyrin (ZnPP) to blood lead levels among children aged 1–3 years in urban North India. In a cohort of 1,246 peri-urban children in Delhi enrolled in a clinical trial, after consent, information about socioeconomic and demographic profile of the household was collected, and detailed physical examination was carried out. All the children were given intervention for 1 year, and at end study,

blood samples were drawn for estimating ZnPP using a fluorometer (Aviv Biomedical, Lakewood NJ) and blood lead levels using atomic absorption spectrophotometer with a graphite furnace (Perkin Elmer Analyst 800). The EDTA blood was analyzed on the same day with Coulter automated hematology analyzer for a detailed hemogram. The mean ZnPP (μmole/g of heme) and blood lead levels (μg/dL) of children were found to be 106.39 and 17.8, respectively. Multiple linear regression analysis was carried out to evaluate ZnPP as a predictor of blood lead levels, and the result indicated that ZnPP was a strong predictor of blood lead levels in children after adjusting for covariates such as age and hemoglobin levels. Blood lead=25.83+0.09×ZnPP−0.15×age−0.31×Hb. Given the good correlation between high blood lead levels and increase in ZnPP, this seems to be a rapid, reliable, and economical screening method for lead intoxication among children in Indian setting.

11.S04

Cadmium reduces beta-casein gene expression in secreting murine mammary epithelial cells

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We have recently demonstrated a significant negative dose–response relationship between beta-casein expression in the lactating mammary tissue and exposure levels of Cd in mice, indicating that Cd may disturb milk synthesis. The mechanism is not known but may involve a direct effect by Cd on the milk producing mammary epithelial cells, as it has also previously been shown that Cd accumulates in the lactating mammary glands and that the element is only poorly distributed into milk. Herein, we have used a murine mammary epithelial cell model (HC11) to investigate the effect of Cd on the secreting mammary epithelium in vitro. HC11 cells were differentiated into a milk protein synthesizing phenotype as demonstrated by beta-casein gene expression. The cells were treated with 0.06, 0.12, or 2.5 μM of Cd, as $CdCl_2$, during differentiation and beta-casein gene expression was measured by quantitative real-time RT-PCR. The levels of ATP in the cells and LDH in the culturing medium were determined at the end of the Cd treatments. Our results show that Cd treatment downregulates beta-casein gene expression in the differentiated HC11-cells in a dose-dependent manner. ATP and LDH levels were not affected at any of the Cd doses examined demonstrating that the reduced beta-casein expression is not caused by a compromised viability or membrane integrity of the HC11 cells. Our results are in line with the hypothesis that Cd disturbs milk protein synthesis in the lactating mammary tissue and indicate that Cd affects beta-casein gene expression in the secreting mammary epithelium directly.

11.S05

Bromide interference with iodine metabolism in the rat

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We studied the effects of an enhanced bromide intake on various aspects of iodine metabolism in the rat. Our studies were performed both under conditions of sufficient iodine supply and of iodine deficiency. We followed the influence of an extremely high bromide intake (>160 mg bromide per animal per day) and also of lower intakes on the uptake of 131I-iodide by the thyroid and by various others organs and tissues, as well as on the kinetics of elimination of iodine from the animal's body. In addition to adult male rats, we also used lactating rat dams and their pups. In the latter animals, we studied the effects of very high bromide intake in the mothers on their performance in the course of the nursing period, on the transfer of iodine and bromide through mother's milk to the suckling and on the prosperity of afflicted young. The results have shown that high levels of bromide in the organism of experimental animals could influence their iodine metabolism in two parallel ways: by a decrease in iodide accumulation in the thyroid, and in the mammary glands in the case of lactating dams, and by a rise in iodine excretion by the kidneys. An increase in the relative weight of the thyroids and a significant decrease in the uptake of radioiodide by the thyroids were found in rats with the highest

bromide intake, especially in those fed the low-iodine diet. Very high intake of bromide in the mothers caused stagnation in the extent of their diet and water consumption in the course of the lactation period, and a striking drop in the production rate of mother's milk. As a consequence, a marked decrease in the body weight increments in their young was observed. In addition, excess bromide in the mothers significantly depressed the extent of iodine transfer from the dams to suckling through mother's milk. We have also proved that bromide, similarly to iodide, readily penetrated into rat milk and, via mother's milk, was transferred in a large extent into the body of the suckling.

11.S06

Trace element status of military personnel after PRT mission

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Military service usually is connected with high psycho-physiological stress and intoxication risk due to various exposures. The exposure to toxic metals along with stress may lead to the zinc deficiency. The aim of the present pilot study was to evaluate the trace elements status (TES) in military personnel after 6 months service in international PRT (province reconstruction team) mission (Afghanistan) and to assess the dietary and behavioral impact on TES in their organism. Questionnaire-based data (29 items), vein blood, hair, and spot urine samples were collected on the second day after return. We evaluated (spectrophotometer and AAS) the enzyme delta-aminolevulinic acid dehydratase (delta-ALAD) activity and concentration of lead, cadmium, zinc, and delta-aminolevulinic acid. Data were treated statistically (EPI-Info package). The participants gave their written consent. Mean (SD) age of 38 participants was 31(6.8) years, 81.6% of those were male. Smoking habits were reported in 60.5%, usage of vitamin supplements—62.9%, additional food supplements—29.4% of servicemen. Mean concentration of trace elements in the organism was found to be within normal physiological range. Concentration of lead and cadmium in smokers vs non-smokers was significantly higher, while the activity of delta-ALAD was lower. The usage of food supplements significantly decreased blood lead level, although the usage was higher among nonsmokers. International military operations were not significant risk factors for environmental exposure to toxic metals. Smoking significantly influenced the changes in TES. Food supplement usage was shown to be preventive factor against environmental and behavioural exposures and maintaining the safe level of essential (zinc) trace elements. Researchers express sincere gratitude to all participants for their contribution to the military health prevention programs.

11.S07

Induction of metallothionein mRNA in the cerebrum and cerebellum after a low dose of thymerosal injection

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Thymerosal, an analogue of ethyl mercury, is used as a preservative agent in vaccines around the world. Mercury has been previously detected in the cerebrum of mouse when a high dose of thymerosal was injected subcutaneously into the mouse with the concentration of mercury in the cerebrum maintained for 1 week. However, mercury was not detected in the cerebrum of the mouse when thymerosal was injected into the mouse using the same quantity as a usual dose for humans. Therefore, it was thought that there was no effect in the cerebrum after thymerosal was injected into man. However, it is possible that a little mercury shifts to the brain and has an influence on it. Here, we observed mRNA level of metallothionein (MT) in the cerebrum and cerebellum of mouse after an injection of thymerosal, as MT is an inducible protein by various stimuli. MT-1 mRNA increased in the cerebrum 6 and 9 h after 12 μg/kg of thymerosal was injected into the mouse. By contrast, the MT-1 mRNA level dramatically increased in the cerebel-

lum 6 and 9 h after the injection. In addition, MT-2 and MT-3 mRNA levels also increased in the cerebellum. An overdose of 6 μg/kg of thymerosal increased the MT-1, MT-2, and MT-3 mRNA levels in the cerebellum at 6 h and increased MT-1 protein level in the cerebellum at 10 h. The present results show that thymerosal may affect the neuron in the cerebellum rather than the cerebrum, even though a low dose of thymerosal is used.

11.S08

SOD gene expression and activities in kidney from rats exposed to cadmium

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The aim of this study was to explore the mechanism of cadmium-induced renal oxidation damage. Rats were given by subcutaneous injection of $CdCl_2$ (0.65 or 2.28 mg/kg), once per day sustaining for 5 days. SOD mRNA expression and SOD activity were determined at different times after termination of treatment, at the same time the cadmium concentration in kidneys were analyzed, and the kidney ultrastructure was examined. The results showed that the mitochondria of renal proximal tubular cells in two treated groups were damaged the earliest, and their SOD mRNA expression level was lower than that in the control group. As for the scanning integral at dot blotting spots, the difference was statistically significant between the high-dose group (35,400± 5,900), the low-dose group (35,919±7,361) and the control group (70,928±7,698). These changes observed in treated groups have a tendency to recover after treatment. In the low-dose group, at 72 h after treatment, the recovery was so good and nearly reached the level as in the control group. The reduction in SOD activity appeared after morphological change and inhibition of mRNA transcription. The authors concluded that inhibition of SOD gene expression may be one of the important factors to cause renal damage by cadmium. Renal damage caused by low dose cadmium with the change mentioned above may be recovered.

Category 12: Trace element detection and quantification

12.P01

Determination of serum/plasma ceruloplasmin and free copper by HPLC/inductively coupled plasma-mass spectrometry for the diagnosis of Wilson disease

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Wilson disease (WD) is an autosomal recessive disorder of copper (Cu) metabolism. It is treatable, but delay of the diagnosis will make the treatment more difficult. The establishment of an analytical method for early diagnosis is effective for early treatment. In the normal subject, 95% of plasma Cu is bound to ceruloplasmin (Cp) and excreted into the bloodstream in the form of Cu-bound protein (holoceruloplasmin). The remaining (free Cu) is loosely bound with albumin and amino acids. In WD patients, Cu is not incorporated into Cp within the liver and not excreted efficiently into bile. As a result, Cu is accumulated in the liver and eventually released into the bloodstream as a free Cu and deposited in the brain, kidneys, and cornea. Finally, the concentration of free Cu is increased in the serum. In the present study, we analyzed the Cu distribution in the serum/plasma by HPLC/inductively coupled plasma-mass spectrometry (ICP-MS) as the new tool for the diagnosis of WD. With this method, Cu was detected at a retention time of 12.2 and 14.0 min in the serum

of a normal subject. The retention time of Cu was 12.2 min, coinciding with Cp standard. In WD patients, no significant Cu was detected within this retention time. These results indicate that a normal level of holo-Cp was not detected in the serum of WD patients using this method. In the case of plasma sample from a normal subject collected with tubes containing ethylenediaminetetraacetic (EDTA)—2Na, Cu was detected at a retention time of 12.2 and 15.6 min. The Cu peak at a retention time of 15.6 min corresponded to Cu standard, which was prepared in the tubes containing EDTA-2Na. This result showed that free Cu was bound with EDTA in the plasma, and clearly detected with this method. We demonstrated here that the analysis of Cu distribution in serum/plasma by HPLC/ICP-MS is beneficial as a novel tool for the diagnosis of WD.

12.P02

Determination of lead and cadmium in various food samples by graphite furnace atomic absorption spectrometry after separation by using the coprecipitation method

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In this study, the coprecipitation method developed using a combination of two-mercaptobenzothiazole (MBT) as a chelating reagent, and copper as coprecipitate carrier was used for the determination of trace lead and cadmium in various food samples by graphite furnace atomic absorption spectrometry (GFAAS). The working conditions of the optimized coprecipitation method were as follows: pH 9.0; the amount of carrier element: 750 μg Cu(II), reagent amount: 1 mL of 1% (*w*/*v*) MBT, standing time: 10 min and sample volume: 750 mL. The method was applied for the determination of Pb(II) and Cd(II) in salami, sausage, chicken, liver, anchovy, spinach, cabbage, leek, onion, dill, parsley, and tea samples. To dissolve the vegetable samples, firstly, concentrated HNO_3 of 65% (*w*/*w*) and then concentrated H_2O_2 of 35% (*w*/*w*) were used. The digestion of the other samples was performed by using mixtures of HNO_3 of 65% (w/w), HClO4 of 70% (*w*/*w*), and H_2SO_4 of 96% (*w*/*w*) acids. After the coprecipitation method was applied, Pb(II) and Cd(II) in the samples were determined by using GFAAS. The matrix modifiers were added as 50 μg $NH_4H_2PO_4$+3 μg $Mg(NO_3)_2$ for both Pb(II) and Cd(II). The signals were measured as peak area. The results of analyses (at mean value, n= 3) for Pb(II) and Cd(II) in the food samples were as follows: 54 and 5.77 ng g^{-1} in the salami, 54 and 3.59 ng g^{-1} in the sausage, 44 and 2.82 ng g^{-1} in the chicken, 0.04 and 0.03 μg g^{-1} in the liver, <DL and 6.04 ng g^{-1} in the anchovy, 3.30 and 0.08 μg g^{-1} in the spinach, 0.87 and 0.05 μg g^{-1} in the cabbage, 57 and 8.70 ng g^{-1} in the leek, 1.59 and 0.06 μg g^{-1} in the onion, 2.12 and 0.07 μg g^{-1} in the dill, 2.54 and 0.05 μg g^{-1} in the parsley, and 0.92 and 0.03 μg g^{-1} in the tea, respectively.

12.P03

Development of an analytical method for the determination of cadmium and lead in offal by Z-ETA-AAS

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Cadmium and lead are present as contaminants in the environment and in foodstuffs and show a great toxicity for humans. Even if the presence of these elements is being constantly reduced through better manufacturing and agricultural practices, the Commission of the European Union (Commission Regulation no. 466/2001; replaced by Commission Regulation no 1881/2006) set maximum levels for these contaminants. As for edible offal of bovine animals, sheep, pig, poultry, the maximum level of cadmium is set at 0.50 and at 1.0 mg/kg wet weight for liver and kidney, respectively; as for lead, the maximum level is set at 0.50 mg/kg *w*/*w*. A method was developed on veal liver spiked at the maximum level of the elements; a microwave-assisted chemical digestion was applied and, then, the analytical solutions were properly diluted and analyzed. The Z-ETA-AAS was chosen to determine the elements of interest. The working temperatures were experimentally set. As for matrix modifiers, mixtures of 0.050 mg $NH_4H_2PO_4$ + 0.003 mg $Mg(NO_3)_2$ and of 0.0025 mg Pd+0.0015 Mg(NO3)2 were used for the determination of cadmium and lead, respectively.

The recovery value at the maximum level was of 101.2% for cadmium and of 101.9% for lead; as for cadmium, the repeatability and within-laboratory reproducibility values were of 3.8 and 3.6%, respectively, and of 2.8 and 3.6% for lead. The linearity range for cadmium was from 0.1 to 5.0 μg/l and from 1.0 to 60.0 μg/l for lead. The expanded uncertainty was calculated for both elements at the maximum level, obtaining the results of 7.5% for cadmium and of 8.6% for lead. The LOQs of the method were of 0.51 μg/kg and 32.0 μg/kg for cadmium and lead, respectively. As for stability studies, the analyses were carried out for a period of 13 weeks. The ruggedness of the method was evaluated by applying the Youden approach. To this end, seven different parameters were chosen and tested.

12.P04

Synthesis and application of a new chelating resin functionalized with salicylaldoxime for the determination of Pb(II), Ni(II), Cu(II), and mn(II) ions in water samples by flame atomic absorption spectrometry

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Amberlite XAD-1180 was functionalized with salicylaldoxime by coupling it through an –N=N– spacer to obtain a chelating resin for separation and preconcentration of Pb(II), Ni(II), Cu(II), and Mn(II) in water samples before the determination by using flame atomic absorption spectrometry. The resulting chelating resin was characterized by infrared spectroscopy and used as solid sorbent. Different factors including pH of sample solution, amount of solid phase, volume and concentration of eluent, sample volume and matrix effects were optimized to obtain quantitative recovery of the analytes. The optimum pH value for quantitative sorption was 6.0, and all the metal ions could be eluted using 5 mL of 1 mol L^{-1} HNO_3. A good relative SD (≤2.1%), quantitative recovery (≥92%), high enrichment factor [100 for Pb and 150 for Ni(II), Cu(II) and Mn(II)], and low detection limits (from 0.2 to 1.6 μg L^{-1}) were obtained. The accuracy of the developed procedure was confirmed by determining the analytes in a certified reference material (TMDA-62, lake water) and analyzing spiked water samples. The proposed method was applied to the determination of the analytes in well water samples with satisfactory results.

12.P05

Effects of formalin fixation on trace element concentrations in biological tissue

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There is frequently a need to preserve biological samples before chemical analysis. A common concern is that reagents, equipment, or the environment may contaminate the samples, resulting in elevated concentrations of analytes. However, the converse may also be true that preservation procedures cause loss of analytes from samples. We have found that storage of human and rat brain tissue in formalin has extracted trace elements from the samples into the formalin solution. Both samples and formalin were analyzed for 20 trace elements employing high resolution ICP-MS. The brain samples had been stored in formalin for different time durations ranging from weeks to decades. We found that for most trace elements, there is a significant leakage from the tissue into the formalin solution, and this leakage increases over time. For some elements, the concentrations in the formalin solution increase more than 100-fold upon long-term storage.

12.P06

The CRL-ISS proficiency tests on trace elements in meat and milk: organization and results

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The CRL-ISS carries out its activity within the Istituto Superiore di Sanità with the institutional task of organising inter-laboratory studies for the National Reference Laboratories (NRLs) of the European Union Member States, to which the control of

chemical elements in food of animal origin such as fish, meat, milk, honey, etc. is requested. Proficiency tests are an important and necessary means to prove the quality of data provided to customers. As these data are often the basis for decision in the testing of official samples, they deserve the utmost attention. As for chemical elements, certified reference materials hardly contain a level corresponding or around the permitted limit (PL); therefore, it was necessary to prepare ad hoc materials having a concentration around it. In this work, the results of 10th PT, related to the analysis of elements such as arsenic, cadmium, and lead in meat and milk, are viewed. Two samples of freeze–dried meat were sent to the laboratories for the first round and two samples of freeze–dried milk for the second. As the natural levels of analytes in fresh samples were below the quantification limit, spiked materials were prepared to obtain levels of elements around PLs in the final samples. As for the results and their statistical treatment, the scanty number of participating laboratories (25) made it necessary to use robust statistics. Among the analysed elements, some anomalous data were observed only for lead and particularly in meat. Considering that criteria used for the choice of sigma targets generated quite small values, the high frequency of robust SDs, lower than such values, shows that precision was the main characteristic of this study, especially for arsenic and cadmium. As for lead, the observed performance has been slightly lower compared with that of other elements, thus confirming the intrinsic difficulty of the analysis.

12.P07

Drinking water: an essential condition for the human existence

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Water is an indispensable factor for the human organism. In natural conditions, water is never found in a pure state; there is always some quantities of dissolved chemical substances or in suspension. The suspension removal is an obligation of water treatment stations, which by treatment procedures, realize a technological flux which assure the drinking water quality in the limits anticipated by the law. The Firiza Lake represents for the moment the alimentation sources of water of the Baia Mare municipal, Romania country. The paper presents studies regarding the water quality from the accumulation lake, characterized by higher turbidity in the studied period and also the water treatment possibilities with new coagulation reagents (aluminium bases polychlorines) and alkalization, compared to aluminium sulphate and calcium hydroxide, following the efficiency and inefficiency of those in the colloidal systems destabilization, and zeta potential reduction. Aluminium-based polychlorines used in the water treatment present the advantage that they react very quickly with the water, assure a good removal of the organic matters from water only by coagulation, leave a small value of residual aluminium in the treated water and correspond to the European requirements. On installation, it can be dosed and also diluted. The experimentally obtained values for the treated water for turbidity, oxidability and residual aluminium, nitrites, and nitrates are in accordance with the raw requirements. The water treatment technique presents the advantage that it can be applied practically on a small automated installation, this being the major reason of this effectuated study. In consequence, the further studies allowed the applying in practice of this treatment techniques at the water drink ability station from the Baia Mare locality in Romania.

12.P08

Iodine speciation in thyroid gland by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry

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A method allowing the determination of iodine species in thyroid gland tissues has been developed. It was based on the coupling of high-performance liquid chromatography (HPLC) with online selective detection of iodine by inductively coupled plasma mass spectroscopy (ICPMS). Experimental conditions

for the extraction, proteolysis, separation, and detection of the iodoamino acids in the thyroid gland were optimized. An efficiency of iodine extraction of 88% from the thyroid gland tissue was achieved using an ultrasonic titanium-point homogenizer. Proteolysis was carried out by incubating the sample with the Pronase E enzyme for 24 h at 37°C in complete darkness. To prevent a high load of organic solvent, from the mobile phase, from reaching the torch, a refrigerated spray chamber was used. This would result in a good stability of the plasma discharge. The experimental conditions were optimized for the determination of different iodine species: iodide (I-), mono-iodine thyrosine (MIT), di-iodine thyrosine (DIT), tri-iodine thyronine (T3), reverse tri-iodine thyronine (rT3), and thyroxina (T4). The influence of the methanol concentration, flow of the mobile phase, methanol gradient, plasma power, flow of nebulizer gas, and dweel time on the retention times (separation) and the signal intensity was evaluated. The relative SD was lower than 8%. Good separation of the iodine–amino acid compounds present in thyroid gland tissues was obtained using the coupling of HPLC-ICPMS. The dominant species in the thyroid gland of healthy individuals are iodide and the species MIT. Di-iodine thyrosine (DIT), T3, and T4 were also detected in a lesser proportion. However, the rT3 species was not detected in these extracts.

12.P09

Field-based arsenic speciation in Argentinean water samples: link to arsenic poisoning

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In recent years, arsenic (As) has become a major concern in terms of its toxicological effects on humans and the environment. Studies are needed to help identify and quantify the levels of As that preside in the environment, particularly the more toxic As3+ species. Development of a field-based method utilizing disposable Bond Elut SPE cartridges incorporating strong cation exchange (SCX) and strong anion exchange (SAX) phases for the determination of As3+, As5+, DMA and MMA has been employed for waters (well, river, tap, and bottled). This methodology enables the separation and preservation of As species in the field with subsequent elution and analysis in the laboratory. Each fraction is analyzed by inductively coupled plasma-mass spectroscopy (ICP-MS) for total As, with correction for the polyatomic 40Ar35Cl+ and validation using CRM's and inter-laboratory comparison data. Separation of As using the cartridges was confirmed by reversed-phase high-performance liquid chromatography (RP-HPLC)-ICP-MS. The field-based method was employed on waters from various rural areas of Argentina (La Pampa, Santiago del Estero and San Juan), where total As levels have been reported to be as high as 8,000 μg/l. High levels of naturally occurring As in groundwater in Argentina have been further affected through extensive mining of gold in areas such as San Juan. In La Pampa, As levels in well water are over 700 μg/l (WHO level for drinking water is 10 μg/l As). Contaminated well waters provide a possible uptake of As into the local populace, potentially leading to chronic As poisoning. Therefore, studies have been carried out on human and animal samples to establish a link between the natural As levels and the current state of health. Human health in regions like La Pampa and Santiago del Estero have reported many cases of skin legions, pigmentation changes, hyperkeratosis, and various forms of cancer associated with the consumption of this water.

12.P10

Multivariate data visualization methods based on elemental analysis of herbal drugs by ICP-MS

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The objective of this work is to demonstrate the power of chemometric methods of analysis to identify possible sources of influence and specific elemental profiles within data set of 45 herbal drugs and their pharmaceutical formulations. All samples were provided from the Serbian market, and before the measurements, microwave-acid-assisted digestion procedure was applied. Complete procedure, from sample preparation, via measurements to the data evaluation was validated according to the rules of

good analytical practice. Having in mind a significant diversity of samples and to highlight complex and high-dimensional quantitative relationships, a common chemometric approach to data analysis was used. Nine elements (Fe, Cu, Mn, Zn, As, Cd, Sn, Hg, and Pb) were chosen as chemical descriptors, and experimental data obtained by inductively coupled plasma-mass spectrometry (ICP-MS) were subjected to multivariate data visualization methods. To identify the patterns and correlations between elements, pattern recognition techniques, such as Principal component and cluster analysis (PCA and CA) were selected as appropriate. When PCA was applied to the autoscaled data matrix, with eigen analysis as initial, three principal components (PCs) were extracted, according to the Kaiser criterion which explain up to 64.30% of variance. Hierarchical agglomerative cluster analysis has shown well-differentiated clusters at significant similarity levels. The features responsible for sample/chemical descriptors grouping refer to the high diversity of herbal drugs and their dosage forms as well as to the elemental patterns obtained within analyzed data set.

12.P11

Methyl mercury in biological fluids by isotope dilution

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Interest in being able to distinguish between different chemical forms of the same element, so-called speciation, is probably most evident in the case of mercury. Nevertheless, the only analytical method for speciation that has achieved international recognition to date is that of the US EPA, Method 1630, for methyl mercury in water. Based on Method 1630 and previous work with whole blood, we have developed methods allowing methyl mercury measurement in human serum and urine samples. These methods feature calibration by isotope dilution, and detection of gas chromatographically resolved mercury species by inductively coupled plasma mass spectrometry, the importance of which will be discussed. Method validation approaches, analytical capabilities, and selected examples from routine applications will be presented.

12.P12

Environmental and health consequences of mercury air pollution

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This paper deals with a fraction of results covering the heavy metal measurement in ambient air by using moss monitoring with special emphasis on mercury. Mercury and its compounds are very hazardous and, even in small amounts, have extremely harmful effect on human health and ecosystem. Atmosphere is an important pathway for mercury cycling in the environment, while bioindicator species (lichens, moss) proved to be effective and efficient tool for mercury effect-related air quality monitoring. Identification and quantification of mercury as atmospheric pollutant in bioindicating organisms is especially important for spatially and cumulative long-term monitoring of mercury concentrations in the air with purpose of reduction and/or prevention of health and ecological risks. During the year 2006, moss samples have been collected on the territory of Croatia to assess deposition and accumulation of air pollutants. The sampling points were located over the entire country, and moss samples will serve as indicators of air quality and environmental changes on the area explored. The samples were analyzed with multi-elemental instrumental neutron epithermal activation analysis and cold vapor atomic absorption spectrometry. According to average mercury concentration 0.067 mg/kg, minimum 0.007 mg/kg and maximum 0.064 mg/kg, the mercury distribution map from the Croatian moss campaign was constructed. This research aims to initiate, develop, and strengthen multidisciplinary research and cooperation between environmental scientists and epidemiologists on the effective application of the moss biomonitoring for air

quality assessment and mercury monitoring as a key for better understanding of the health and environmental risks and consequences of the mercury air pollution.

12.P13

Pre-analytical factors can cause intermittent raised urine cadmium results

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To identify the cause of raised urine cadmium levels (>18 nmol/L for nonexposed workers) found in 20 out of 36 medical imaging workers using Cerrobend (Pb 27%, Cd 10%, Sn 13%, Bi 50%), urine cadmium is measured on a Varian220Z Graphite Furnace Atomic Absorption Spectrophotometer with Zeeman background correction. Serial urine samples were collected from exposed and nonexposed workers at the same site. As it was observed that the collection pottles referred from this site had yellow tops, in contrast to the pink topped pottles routinely used in our laboratory, the two types of pottles were tested for contaminating cadmium. A sample (100 ml) of normal urine was added to the pottles, mixed by inversion and analyzed the following day. During the same period, only three out of 29 urine samples from other workers involved in routine occupational monitoring at other sites had raised cadmium. Investigations also showed that many nonexposed workers from the original site also had intermittently raised urine cadmium levels, and repeat testing showed that the raised urine cadmium results from the Cerrobend workers were inconsistent and not related to the degree of exposure. This suggested that pre-analytical factors were involved. Urine stored overnight in the pink pottles had cadmium levels of <5 nmol/L, but samples from the yellow pottles were consistently >61 nmol/L. Two other batches of yellow-topped urine pottles in use at this site were similarly investigated—with only one batch showing contamination. On contacting the manufacturer, it was disclosed that uncontrolled amounts of cadmium sulfide pigment and recycled plastic was used to make the yellow lids, and this varied between batches. Exclusion of pre-analytical contamination by following collection protocols specified by the analyzing laboratory and checking containers before use, may prevent workplace anxiety and further unnecessary and costly investigations.

12.P14

Novel device for direct measurement of non-ceruloplasmin copper in Wilson's disease

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Wilson's disease (WD) is a rare autosomal recessive genetic disorder which causes elevated copper levels in blood, urine, liver, and brain. Normally, total serum copper levels range between 70 and 150 μg/dL and 75 to 95% of the total is associated with copper tightly bound to the serum protein ceruloplasmin (Cp). The remaining copper is known as non-ceruloplasmin-bound free copper (NCBC). In WD, NCBC elevation is in part diagnostic and is thought to play a role in the pathophysiology of the disease. The current method (subtraction method) to determine serum NCBC involves two independent measurements. First, total serum copper is measured by atomic absorption, and second, serum Cp is measured by immunoassay. The concentration of Cp (mg/dL) is then multiplied by 3 μg/mg to estimate the concentration of Cp-bound copper (μg/dL). NCBC is calculated by subtracting Cp-bound copper from total copper. This method has a number of limitations. It is indirect, expensive, slow, and incapable of distinguishing Holo- from Apo-Cp. Due to these limitations, we developed a simple, economical, rapid, and direct method using a portable point-of-care device which measures NCBC and total serum copper. The device (FreeBound®) measures these two forms of serum copper based on a proprietary process incorporating anionic stripping voltammetry. Plotting current vs potential provides a peak specific to copper, and the height of this peak is proportional to the amount of copper in serum. Most importantly, the device measures NCBC apart from Cp-bound copper based on the kinetics of deposition. We compared NCBC values by FreeBound® to those found by the "subtraction method" in serum samples from WD patients. The correlation of determination between the

methods was very good at r^2=0.93. Similar results were found for total copper. Total copper and NCBC were also used to calculate serum Cp. In conclusion, this new device gives rapid, inexpensive, and direct detection of NCBC at point-of-care.

12.S01

Separation of proteins in cerebrospinal fluid by size exclusion HPLC and determination of trace elements by HR-ICP-MS

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The central nervous system is surrounded by cerebrospinal fluid (CSF). CSF provides a biological matrix for trace element analysis reflecting the living brain. A method to study the protein binding patterns of trace elements in human cerebrospinal fluid (CSF) is described. By employing size exclusion chromatography combined with high performance liquid chromatography (SEC-HPLC), proteins in CSF samples were separated according to size. The column was calibrated to separate proteins in the molecular weight range 6–70 kDa, with special emphasis on metallothionein. Fractions were then analyzed off-line for trace elements using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). The separation technique developed together with HR-ICP-MS analysis is very useful for studying metal-containing proteins in body fluids when metal concentrations are very low, which is the case after fractionation of CSF by HPLC. We were able to accurately determine more than ten elements of clinical interest in the CSF fractions. Results are presented for Cd, Mn, Fe, Pb, Cu, and Zn. The total concentrations of 16 trace elements in human plasma and CSF are also presented.

12.S03

Very accurate (definitive) methods by RNAA. The idea and recent achievements

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The idea of very accurate (definitive) methods by radiochemical neutron activation analysis (RNAA) for the determination of selected trace essential and/or toxic elements in biological materials is briefly presented. Definitive methods by RNAA rely on combination of a very selective and quantitative postirradiation separation of a given radionuclide from essentially all accompanying radionuclides followed by γ-ray spectrometric measurement. The methods elaborated so far are briefly mentioned. A new method for the determination of selenium employs cation exchange and extraction chromatography (3,3'-diaminebenzidine supported on XAD4) columns for selective and quantitative isolation of 75-Se which is then measured on the resin by γ-ray spectrometry. New method for arsenic uses specially prepared cation exchange resin column in the ferric iron form for the selective separation of 76-As. The performance of the methods is demonstrated by the analysis of several certified reference materials (CRMs). Definitive methods are used mostly for verification of accuracy of other methods of inorganic trace analysis and for certification of the candidate reference materials.

12.S04

Ion spectrometry (SIMS) and electron microscopy (TEM) correlative imaging in the quest of comprehensive trace element localization at subcellular level, in normal and pathological keratinocytes

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Due to a sensitivity close to 1 ppm, SIMS imaging has made currently possible localized analysis of trace elements at tissular level. Improvements in spatial

resolution permitted a better recognition of trace element cellular targets. In spite of a relative high resolution (50 nm), dynamic SIMS imaging remains far behind TEM, and it is still difficult to identify with certainty the various cellular compartments observed with SIMS imaging. The use of these two complementary methods, in terms of correlative imaging, was applied to the follow-up of keratinization process in normal and pathological cultured keratinocytes before trace element localized analysis. The culture medium contained lithium gluconate. First attempts were performed on serial sections: ultrathin sample sections (60 nm) for TEM and thin sections (1 μm) for SIMS. More accurate correlations required the study of a same sample section, both with TEM and SIMS instruments. So, a slight modification of SIMS sample holder was performed and allowed a direct analysis of sample sections (200 nm) when deposited on the grid previously observed by TEM. A specific treatment of the sample avoided undesired charge accumulation. Positive and negative secondary ion images were performed. S- images were helpful for mapping the sulfur-rich precursor of keratin and interpreting keratohyalin compartment observed by TEM, in normal and pathological cells. The correlation with Li+ distribution was established. Such SIMS and TEM new approaches are promising for comprehensive biodistribution of trace elements at subcellular level, through physiological and pathological process.

12.S05

Recent developments in ICP-MS applied to studies in nutrition and health

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Inductively coupled plasma mass spectrometry (ICP-MS) with sample introduction via nebulization of aqueous sample solutions is now extremely well established for the high throughput multielemental and isotope ratio (IR) analysis of diverse geological, biological, and clinical matrices, with detection limits typically at ng g^{-1} concentrations, and under favorable circumstances, down to fg g^{-1} levels, and a dynamic range of up to ten orders of magnitude. The widespread use of high-resolution spectrometers permits the determination of biologically important elements such as As and Fe, for example, which are difficult to determine using conventional quadrupole instruments owing to polyatomic interferences ($^{40}Ar^{35}Cl+$ and 40Ar16O+, respectively). Multi-collector instruments have allowed improved IR analyses, for example, of 54Fe, 56Fe, and 57Fe in human blood, liver and muscle. Another area of rapid growth over the last decade has been the coupling of ICP-MS to diverse chromatographic separations permitting, for example, the determination of species such as As(III), As(V), and dimethylarsinate in angiosperms using ion chromatography-ICP-MS. In addition to these advances, improved laser ablation technology for solid sample introduction to ICP-MS now permits studies requiring spatial resolution of a given sample. For example, Zn, Pb, Mg, Fe, Sr, and Cu were recently monitored in human teeth using ablation Q-switched pulses from a Nd:YAG laser quadrupled to a wavelength of 266 nm. Such measurements are useful as a record of health status and exposure to pollutants. Laser ablation is also now being applied together with gel separation techniques to examine, for example, Fe, Cu, and Zn associated with brain proteins separated by (2D) gel electrophoresis. These and other significant recent developments will be critically reviewed.

12.S06

Elemental analysis of biological materials—state of the art

Douglas Baxter, and Ilia Rodushkin
ALS Scandinavia (Luleå, Sweden)

After a decade of research into physiological levels of elements, a single analytical technique has emerged with the requisite capabilities. Inductively coupled plasma sector field mass spectrometry (ICP-SFMS) permits more than 70 elements to be determined at both normal and elevated concentrations, fulfilling a variety of needs in the fields of nutrition, pharmacology, health care, and toxicology. Here, application of ICP-SFMS to the analysis of human whole blood, serum, and urine from unexposed individuals will be described, with emphasis on quality control routines. Examples will be given to illustrate some of the pitfalls that can be avoided using high instrumental

mass resolution in the analysis of biological materials. Finally, a database compiling baseline concentrations of over 70 elements, as determined by ICP-SFMS in human biological fluids, will be presented.

Category 14: Invited speaker topics

Keynote address

14.1.1
Mediterranean diet, traditional foods and health: critical components and mediating mechanisms

Antonia Trichopoulou
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Nutritional investigations have provided strong indications that a diet that adheres to the principles of the traditional Mediterranean one is associated with longer survival. This could be partly attributed to Mediterranean traditional foods, which this diet implies. Traditional foods are an integral part of the Mediterranean diet, and there is a need to investigate them to elucidate their role in the beneficial effects of this diet. There is also a need to study traditional foods with a view to enrich and improve our diet and at the same time preserve important elements of our cultural inheritance. Our research team has been working towards this approach with the objective to formulate a multifaceted framework for the systematic investigation of traditional foods and recipes, aiming primarily at the elucidation of the role of traditional Greek diet on health. The study of traditional foods in Greece started in 1992 and has several aspects, including the determination of their nutrient and non-nutrient value. This multifaceted methodology is currently being expanded to 12 European countries. The analysis of several traditional Greek foods indicated that the majority of them are rich in antioxidants and several trace elements and meet current criteria for a prudent diet. Therefore, the traditional foods of Greece may contribute to the apparent health benefits of the Mediterranean diet. Likewise, the analysis of traditional foods in other European countries may reveal beneficial health effects in some or many of them, as tradition rarely, if ever, honors foods and diets incompatible with good health.

Trace minerals: modulators of arterial function

14.1.2
Manganese: modulator of vascular function, structure, and metabolism

Dorothy Klimis-Zacas[1], and Anastasia Kalea[2]
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Manganese (Mn), has been reported to influence the genesis and development of cardiovascular disease by participating as a cofactor of several enzymes that protect cells from oxidative stress and aid in the synthesis and maintenance of connective tissue extracellular matrix and structure. In addition, Mn modulates important intracellular pathways, by activating proteins for cell-signaling, influencing cell surface receptor binding and adhesion, and interacting with ionic-channel receptor complexes. We have documented that Mn is involved in arterial glycosaminoglycan metabolism (GAG) by affecting the total proteoglycan content of the aorta, altering the concentration and molecular structure of arterial heparan sulfate. Manganese supplementation resulted in reduced sulfation of heparan sulfate proteoglycans (HSPGs) and altered galactosyl transferase activity. The reduced expression and undersulfation of HSPGs with Mn supplementation may indicate a decreased ability of vascular cells to interact with biologically active molecules, such as lipoproteins and thus protect the endothelium from lipid deposition and lipoprotein oxidation. Alterations in cell membrane binding to a variety of extracellular ligands may affect signal-transduction pathways and arterial functional properties. We have documented that the presence of dietary Mn suppresses vasoconstriction induced by an alpha-adrenergic agonist and operates through an endothelium-mediated pathway. We also studied the effect of Mn on endothelium-dependent (Ach-induced) and endothelium-independent (SNP-induced) vasodilation as it relates to vascular function and reported that Mn operates in the cyclooxygenase pathway by inhibiting the synthesis or activity of a prostanoid-derived vasoconstrictor present at basal and stimulated levels. Thus, Mn is crucial in atheroprotection and maintenance of vasomotor tone with implications on cardiovascular disease.

14.1.3
The role of copper in nitric-oxide-mediated vasodilation

Dale Schuschke
University of Louisville School of Medicine (Louisville, USA)

Dietary copper and cuproenzymes have a significant role in microvascular control mechanisms. Of particular importance is the endothelium-dependent regulation of arteriolar vascular smooth muscle. We have used the rat cremaster muscle model to study the in vivo microcirculatory effects of dietary copper. Our initial results showed that nitric oxide (NO)-mediated dilation is attenuated in microvascular resistance vessels during dietary copper deficiency. This attenuated dilator response is accompanied by an inhibition of copper–zinc superoxide dismutase (Cu,Zn-SOD) activity, and the decreased dilation is reversed by the addition of exogenous Cu, Zn-SOD. We have proposed that when this cuproenzyme is inactivated by copper restriction, there is a buildup of superoxide anion (O_2^-), which then reacts with endothelial cell NO to partially destroy the NO available for diffusion to the vascular smooth muscle. This hypothesis is supported by the observation that during copper deficiency, there is an increase in peroxynitrite (ONOO–), the end product of the NO–O_2– reaction. Peroxynitrite is also a known inhibitor of endothelial cell Ca^{2+} mobilization, which is required for endothelial NO synthase (eNOS) activity. In a study of endothelial cell NO production, we have shown that acetylcholine-induced Ca^{2+} mobilization and NO release are both attenuated in resistance arterioles of copper-deficient rats. These results suggest that during copper deficiency, there is not only a build up of O_2^- which scavenges available NO, but there is also an additional inhibitory effect of elevated ONOO– to reduce NO synthesis.

14.1.4
Selenium status and regulation of vascular homeostasis

Lorraine Sordillo
Michigan State University (East Lansing, USA)

Evidence suggests an inverse relationship exists between selenium (Se) nutrition and cardiovascular disorders. Several intervention studies are currently underway to assess the benefits of Se supplementation to control a wide variety of conditions in which oxidant stress and inflammation are the predominant pathological features, including atherosclerosis. Earlier epidemiological and ecological studies examined the potential benefit of Se administration to control cardiovascular disease; however, they have proven to be inconclusive. A number of underlying factors exist to explain some of the equivocal finding from these prospective studies including identification of specific cellular processes that are affected by Se status and determination of the specific Se-dependent bioactive components that are responsible for the desired response in targeted cellular environments. The importance of Se to health may be related to selenoprotein activities, such as glutathione peroxidase and thioredoxin reductase that have diverse biological roles. These enzymes can function as potent antioxidants by directly reducing pro-atherogenic reactive oxygen species to less reactive metabolites. Recent evidence also suggests that certain selenoproteins are capable of modifying cellular responses to oxidant challenge by controlling the balanced expression of cytoprotective and proinflammatory factors. This paper will define the regulatory role of individual selenoproteins in orchestrating the expression of pro-inflammatory genes during oxidant stress through their ability to control redox-sensitive signaling events. The long-term implications of this work will be the ability to identify foods that can provide the optimal amount of specific biological active selenoproteins needed to control the development of atherosclerosis and other cardiovascular disorders.

Raulin Award Lecture

14.2.1
Zinc nutriture and the fetal origins of disease

Harold Sandstead
The University of Texas Medical Branch (Galveston, USA)

Primary zinc (Zn) deficiency, “highly unlikely” in 1959, now common, impairs gene expression and health of progeny. The essentiality of Zn for reproduction is

known from experiments in model systems that began more than 50 years ago and observations on humans that began more than 30 years ago. From this background, it is clear that Zn deficiency increases risk of abortion, teratology, fetal-stunting, prematurity, and residual functional abnormalities. That there is a problem in the USA, is suggested by the similar associations of iron (Fe) and Zn nutriture with food choices; the strong association between serum ferritin concentrations and Zn nutriture, measured by Zn kinetics; and the >25% prevalence of absent Fe stores, measured by serum ferritin concentrations, among US premenopausal women of child-bearing age. Independent of knowledge of Zn, many epidemiological observations found associations between birth weight, and more recently, maternal nutrition and the health of progeny in later life. Metabolic imprinting that affects later health involves epigenetic mechanisms. Zinc is one of several micronutrients essential for these phenomena. Given the world prevalence of Zn deficiency, conservatively estimated at 20.5%, the contribution of developmental Zn nutriture to human health in later life begs clarification and application. At the same time, research is needed for the understanding of how Zn nutriture affects metabolic imprinting in isolated cells, other species, and humans.

Trace element nutrition and dietary recommendations

14.2.2
The relevance of trace element nutrition in human health

Sophie Ermidou-Pollet[1], and Serge Pollet[2]
[1]University of Athens (Nea Makri, Greece); [2]HTES (Athens, Greece)

The relevance of trace element nutrition in human health is obvious from the beginning of the life until the old age. Proper trace element nutrition during pregnancy may be important for maternal health and foetal growth and development. Growth of infants and children is a complex process in which several nutritional factors, including trace elements, play roles and interact, and the extent and consequences of such interactions are still not completely understood. Review of the literature has shown that trace element intakes are particularly important in the prevention of cancer, cardiovascular diseases, atherosclerotic diseases, osteoporosis, age-related ocular diseases, and in aging. Further research is required to determine the optimal micronutrient combinations and the doses required according to timing of intervention. For long, it was not fully accepted that food can have an influence on brain structure and, thus, on its function, including cognitive and intellectuals. In fact, trace elements have been directly evaluated in the setting of cerebral functioning. Finally, deficiencies of trace elements can have profound effects on the development, health, and well-being of human subjects. They may have various etiologies: inadequate intake, inherited genetic disorders, malabsorption due to intestinal pathology or reduced bioavailability, excessive urinary, and/or fecal excretion. Although some progress has been made in the understanding of dietary interactions that determine biological availability of trace elements, a basis for quantitative treatment of these interactions is still lacking. Trace element supplementation for meeting the requirement is also not so easy to perform. Interactions with other minerals or dietary constituents need consideration in the evaluation.

14.2.3
International dietary standards for trace elements

Jean Freeland-Graves, and Zhaoli Dai
The University of Texas at Austin (Austin, USA)

The essentiality and toxicity of trace elements in the human diet have been estimated to be related to mortality and morbidity in over half the world's population. Problems may arise from inadequate and/or poor quality food supply, cultural and societal norms, and environmental factors such as geography, contamination, parasites, and political and economic situations. In addition, trace element status is influenced by impairments in metabolism (absorption, digestion, transport, utilization, and excretion) and consequences of diseases and chronic health conditions. This impact of microminerals on health and society necessitates the formulation of recommendations of dietary standards that can be disseminated to health professionals, consumers, researchers, and agricultural and government agencies and policy makers. Common approaches to assess trace element status have included clinical consequences, nutrient balance, functional indicators (biomarkers), and pop-

ulation-based assessments of nutrient intakes associated with good health. Yet, the vast diversity of countries, populations, and diets throughout the world make it difficult to identify the most appropriate values for each segment. Priorities among regions of the world may differ, from elimination of hunger and malnutrition and maintenance of biological functions to prevention of chronic diseases via optimal intakes. Variations due to genetic determinants, body composition, stages of life, and lifestyle may further complicate recommendations. Clearly, further studies are warranted to develop and compare the multiplicity of dietary standards on a global basis.

14.2.4
Future dietary standards for trace elements

Lindsay Allen
USDA, ARS (Davis, USA)

Since the dietary recommended intakes for most trace elements were released for the United States and Canada (FNB/IOM, 2000), information has been acquired that may improve our estimates of future dietary standards for some, but not all, of these nutrients. In the case of iron, there is considerable current interest in potential adverse effects of higher intakes in the form of supplements, which could change the current criteria on which the UL is based. Recommended iron intakes for infants should be increased based on lower estimates of iron absorption from breast milk, and there are new data showing that cutoffs for iron status indicators in infants should be changed. The International Zinc Nutrition Consultative Group (IZiNCG) has published a detailed review of recommended dietary zinc intakes, which concludes that zinc requirements are substantially lower than those published by IOM/WHO for several age groups, based primarily on using a different set of studies for estimating endogenous zinc losses. A new model is available for predicting the effects of total dietary zinc and total phytate on zinc absorption. However, there has been relatively little progress finding better indicators of human zinc status. New data from long-term feeding of a high copper diet suggest that the current upper level for copper is too high and should be lowered. Ongoing research is investigating the effect of iron deficiency on copper absorption. WHO has recently increased recommended iodine intakes during pregnancy, a position that has been endorsed by the American Thyroid Association. Regarding silicon, new data show that there is a significant correlation between silicon intake and urinary silicon, which may provide some information on which to estimate requirements in the future.

Trace element speciation

14.3.1
Challenges in speciation of workroom air exposures

Yngvar Thomassen
National Institute of Occupational Health (Oslo, Norway)

Most compounds of the elements can occur in different physical states, namely, solid, gas, liquid, vapor, or aerosol. Each of these states can influence or determine the nature of exposure, the uptake or absorption, and the toxicological consequences. Many workplace and atmospheric aerosols are composed of a complex mixtures of chemical compounds. As a number of factors are involved in determining toxicological responses to the chemical exposure, this presentation will illustrate how modern technology used in aerosol measurement and characterization can provide more detailed information about the specific particle-size distribution, time-resolved concentration monitoring of particle-size fractions, chemical composition, and morphological characteristics of workroom aerosols collected in metal refineries. Such knowledge would improve our understanding of the basis for the reported differential health risks. Exposure to, e.g., nickel oxides and sulfides, which are relatively insoluble in water, figures prominently in the epidemiology of nickel-related lung and nasal cancer. The carcinogenic potency of water-soluble nickel compounds and metallic Ni continues to be debated. For exposure assessment of workers in ongoing epidemiological studies, novel chemical leaching schemes have been developed for bulk speciation of nickel and manganese compounds likely to be present in nickel and manganese alloy smelters. Furthermore, single aerosol particle characterization of aerosols collected from such metal smelters has been performed using modern methods of analysis to obtain deeper understanding of particle compositions and physiological behavior. The aim of this presentation is to discuss the determinant factors and their measurements judged

to be important in the understanding and rationalization of the biological chemistry and toxicity of inorganic compounds of the elements with illustrations from a variety of occupational environments.

14.3.2
Analytical challenges to the human trace element speciation

Ryszard Lobinski
Equipe de Chimie Analytique Bio-inorganique (Pau-Pyrénées, France)

The measurement of the total trace element concentrations is widely used to assess the elemental status in humans. The recent advances in analytical methodology allow, however, the access to information on the concentration levels of the individual species of an element, referred to as speciation. In particular, the progress in inductively coupled plasma mass spectrometry (ICP MS), used as a detector in chromatography and capillary and gel electrophoresis, have resulted in a considerable number of in vitro and in vivo studies of interactions of trace elements in biomolecules. The multi-isotopic (including nonmetals such as S, P, or Se) detection capability, high sensitivity, tolerance to matrix, and large linearity range regardless of the chemical environment of an analyte make ICP MS a valuable complementary technique to electrospray MS and MALDI MS. The lecture discusses the possibilities of the state-of-the-art hyphenated techniques to the trace element speciation in biological samples of human origin. The examples chosen illustrate, in particular, advances in the three major areas: (1) monitoring of the metal–protein interactions and the determination of metal–protein complexes in vitro and in vivo by hyphenated techniques using ICP MS detection in liquid chromatography, gel electrophoresis, and capillary electrophoresis; (2) specific determination and screening for selenium-containing- and selenoproteins and selenoamino acids in blood and serum using two-dimensional affinity chromatography and size-exclusion chromatography with ICP MS detection, and (3) in vivo monitoring of the metabolism of metallodrugs containing covalently bound Pt or Ru atom with a focus on ultratrace (0.1 ng/ml detection limits) quantification of the initial drug and the products of its metabolism.

14.3.3
Studies on the biotransformation of arsenic and selenium in humans

Kevin A. Francesconi
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Arsenic and selenium are two metalloids which, in their inorganic forms, have interesting similarities and differences in terms of their toxicity, biotransformation, and excretion in humans. Both elements are readily methylated in the body, presumably as a detoxification strategy, and the methylated forms are quickly excreted mainly in the urine. In the case of selenium, the common urine metabolites are methyl selenosugars and trimethylselenonium ion, whereas arsenic metabolic products are dominated by simple mono- and dimethylated derivatives. Because selenium is incorporated into essential biomolecules, and only the excess is excreted, the formation of the urine metabolites is strongly dependent on dose and becomes significant only at higher exposure. For arsenic, which has no established essential role, the pattern of urinary metabolites is more constant, and the quantity excreted reflects exposure, even at lower exposure levels. Humans, however, can also show considerable individual variability in the way in which they biotransform both elements. Understanding the reasons for this variability may help explain the observed effects, both beneficial and detrimental, of these elements in population-based studies. Recent results will be discussed from investigations with humans after exposure to inorganic and organic forms of arsenic and selenium.

14.3.4
Purification and identification of low molecular fraction of selenium species in liver cytosol by high-performance liquid chromatography and tandem mass spectrometric techniques

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Selenium (Se) is a biologically intriguing element. It is essential but also can be toxic to animal and human health. In the mammalian body, selenium exerts its biological functions through its incorporation into

selenoproteins as selenocysteine. Characterization of selenium species (selenoproteins, Se-containing proteins and other species of low molecular weights) in samples of mammalian origin is one of the major fields of interest in selenium speciation research. This study aims to establish a methodology based on the mild sample preparation procedures, multiple dimensional high-performance liquid chromatographic (HPLC) separation/purification and tandem mass spectrometric (MS/MS) detection for the identification of Se species in the low molecular weight (LMW) fraction of porcine liver cytosol. Liver contains complex biological matrix in which Se biomolecules and its other species are present at low concentration levels. In such a condition, sample preparation and purification are crucial procedures to the successful mass spectrometric identification of these molecules. For this purpose, a subcellular fractionation protocol was used to prepare the liver cytosol. Subsequently, the LMW fraction of Se species was collected offline according to the Se elution profile obtained from the size exclusion chromatography hyphenated with inductively coupled plasma mass spectrometry (ICP-MS). This LMW fraction was further purified by a reverse phase (RP) column. The identities of Se species was investigated by RPHPLC-ICP-MS and by using RPHPLC coupled with electrospray ionization and/or atmospheric pressure chemical ionization MS/MS. In our study, besides selenomethionine and methyl 2-acetamido-2-deoxy-1-seleno-beta-D-galactopyranoside (SeGalNAc), more than three other Se species were found in liver cytosol. Our efforts towards the identification of these unknown hepatocytosolic Se species by LC-MS/MS techniques will be discussed.

14.3.5

Arsenic biomonitoring in children playing on chromated copper arsenate-treated playgrounds

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One industrial application of arsenic is its use as a wood preservative in the form of chromated copper arsenate (CCA). The widespread use of CCA-treated wood in playgrounds has generated public concern over the safety of the application. The high hand-to-mouth frequency of children may increase their risk of ingesting arsenic during contact with CCA-treated playgrounds. Our group has previously measured the arsenic concentrations in the hand wash samples of children playing on CCA and non-CCA playgrounds. Arsenic levels were four times higher in the hand wash samples from children playing on CCA playgrounds. However, it is not known how much arsenic on the children's hands is ingested or whether this amount contributes substantially to the overall internal exposure levels. The objective of this study was to determine children's exposure to arsenic by speciating and quantifying arsenic in urine and saliva samples of children in contact with CCA and non-CCA playgrounds. We collected one pre-play saliva sample, and three post-play samples each of urine and saliva. Arsenic analysis was conducted using HPLC-ICPMS. The arsenic species detected in the samples were inorganic trivalent and pentavalent arsenic, dimethylarsinic acid, and monomethylarsonic acid. There were no considerable differences in the speciation patterns or total arsenic concentrations in the urine and saliva of the two groups. The mean total urinary arsenic was 16 μg/L in the CCA group and 13 μg/L in the non-CCA group (p=0.5046). The mean total salivary arsenic was 0.94 μg/L in the CCA group and 1.2 μg/L in the non-CCA group (p=0.4859). As the concentration and speciation of arsenic in the samples from children in contact with CCA and non-CCA playgrounds were similar, we conclude that contact with CCA-treated playgrounds does not considerably contribute to the total ingested arsenic. However, we recommend that children wash their hands after contact with CCA-treated playgrounds.

Health consequences of trace element deficiencies

14.4.1

Trace element nutrition: a neglected health issue in developing countries

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According to a recent report by the World Health Organization (WHO), deficiency of trace elements is

widespread, but unrecognized. Nearly a third of the current population of the world suffer from the deficiency of iron and iodine alone. In countries with low socioeconomic status, it is very likely that deficiencies of other trace elements such as zinc and selenium are very common. A lack of characteristic symptoms and lack of appropriate diagnostic tools are the major reasons that such deficiencies are not recognized. The fourth leading cause of death in many parts of the world in the 1990s was due to diseases related to nutritional disorders. Even the fourth projected cause of death in the coming years would be due to nutrition-related disorders. In spite of this report by the WHO, trace element nutrition as a health issue has a very low priority in most developing countries. In the present study, we have investigated the dietary patterns in the general population of 13 countries. Daily dietary samples from 13 countries, both developed and developing, were collected by a standardized food collection technique. After proper handling of the diets, the contents of major and minor trace elements were analyzed by modern techniques. Blood samples were collected from adults and children in India, Pakistan, Sweden, and France to analyze the lead content. The results showed that the intakes of potassium, magnesium, zinc, iron, and selenium were low when compared with the recommended levels (RDA) at the time of investigation. The levels of lead in the blood of children in India and Pakistan were three to five times more than the levels observed in Sweden. The exposure to other heavy metals such as cadmium and mercury varied to a great extent in different countries. The implication of these findings will be discussed from a public health point of view.

14.4.2

Studies on the placental and mammary gland transfer of trace elements: impact of possible trace element deficiencies in infancy

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To evaluate the barrier function of the human placenta at the end of gestation, the transfer of 17 trace elements from the mother to the foetus was investigated. To this end, 29 maternal and corresponding umbilical cord sera (UCS) were considered. In addition, the uptake of trace elements by the foetus was evaluated in nine pairs of arterial and venous UCS. Our results indicate that the placenta activates the transfer of selected elements such as Mn 150%, Zn 148%, Ca 120% and Mg 105% compared to the maternal serum content, i.e., 100%. Yet, the placenta can also inhibit the transfer, for example, of Cu (20%), Pb (50%), Se (55%), and Co (60%). However, the placenta is permeable for Li, Mo, and Sr after a concentration gradient and resulting in comparable elemental concentrations in maternal serum and UCS. Our findings also indicate that the placenta may act as an organ of excretion to maintain homeostasis between Zn and Cu. Determination of 14 trace elements in 27 maternal sera and corresponding colostrum samples revealed an activating and an inhibiting effect of the mammary gland for several trace elements. In colostrums, the concentration of Zn was 15 times higher than in maternal sera. For Mo, Mn, Sn, and Ca, these values amounted to 8.1, 2.8, 2.3, and 2.2. An inhibiting transfer effect was observed for Se (47%) and Cu (19%). A concentration gradient mode of action was established for the transfer of Li and Sr. The intensive transfer of Zn by the mammary gland during the period up to the third month postpartum results in high concentrations of Zn in mother's milk. This is obviously designed to cater for an increased need for Zn of newborns and young infants. One may speculate that this period of a high Zn supply may not be sufficiently long to meet the requirements of premature infants. Hence, it may cause Zn deficiency in these infants when exclusively breast-fed.

14.4.3

Trace element deficiency treatment: much more than supplementation with deficient trace element

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Maintaining optimal mineral balance is of great importance in the prevention, mitigation, and treatment of numerous diseases. Recent data on nutrient and supplement intake indicates that the vast majority of people suffers from micronutrients malnutrition. Investigations of trace elements in blood and scalp hair conducted on healthy adults from different countries showed that most of tested participants have

reduced concentration of at least one element and disturbances in the ratios of many other elements. To improve deficient trace element intake, humans use various form of supplements and/or foods with improved nutrient status. To identify the most desirable strategy for reducing risks from severe selenium (Se) deficiency and, in lower extent, other nutrient deficiency in the food chain, we tested efficiency of dietary supplements with Se in the form of selenite and the Se-enriched brevery and bakery yeast, and crops biofortified with Se use in human and animal nutrition. Conducted investigations on humans and animals showed that in raising deficient trace element level, any form of dietary supplement was not so efficient as food that contains trace elements in natural forms and beneficial amounts. Such food represents a well-balanced source of deficient trace elements and has optimized content and composition of wide range nutrients important for the proper functioning of the body. Offered strategy cannot provide enough trace elements when individual requirements for same the nutrients are increased. In such cases, to determine individual needs for trace elements, recognise symptoms of mild trace element deficiency, and determine the optimal dose and combination of trace elements and other nutrients, we utilized information that offer hair analysis to trace elements, saliva pH measurement, and bioinformatical methods. Health effects of such formulated nutritional supplements used overcame all our expectations.

14.4.4

Predicting relative concentrations of bioavailable iron using in vitro approaches

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There is an unequivocal need for predicting the bioavailability of dietary iron. Bioavailability is a key concept in iron nutrition. Ranging from 1 to 20%, it is the limiting factor in dietary iron intake, and consequently, it plays a central role in the primary prevention of iron deficiency. Many studies have identified a variety of dietary factors that enhance or inhibit iron bioavailability. Nevertheless, new measurements and/or prediction of iron bioavailability are continuously required because of new developments in the science of foods and nutrition. For example, interest in novel food ingredients (e.g., sterols, milk peptides) or in new fortified foods (e.g., milk products, gluten-free breads) or in local, traditional, and ethnic foods, have produced a need for new measurements of iron bioavailability. Consequently, there is a continuing need for establishing simple, reliable screening techniques that would allow the comparison of a large number of samples. In vitro procedures, in cell-free or cell model systems, offer advantages but also present limitations. New developments in the use of the in vitro methodologies and examples from the application of these methodologies will be discussed.

14.4.5

Iodine in the food chain of animal and man: intake, balance and requirement

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The iodine intake of seven omnivorous and ovolacto-vegetarian women and men was examined in 21 test teams in Central Europe and Mexico by the duplicate portion technique over seven successive days. Women in Germany presently consume >100 μg, men >120 μg I/day on average, or 1.3 and 1.5 μg/kg body weight/day, respectively. In Mexico, I intakes of women and men amount to 150 and 200 μg I/day or 2.5 and 2.6 μg I/kg body weight and day, respectively. Although on average, the normative iodine requirement of adults is met (1 μg, recommendation 2 μg, per kg body weight and day), 25% of German adults take in <100 and 120 μg I/day, respectively. Iodine consumption in summer is 40% lower than in winter. It increases by 30% with increasing age (20–69 years) and 45% with increasing body weight. In the endemic iodine- and selenium-deficient region of Central Europe, where iodine intake and serum T4 status were normalized by iodinated salt, it was not possible to normalize the free T3 level in the blood serum. The GSH-Px levels of women's blood serum (170 U/L) were normal. Supplementation of 50 μg Se and 100 μg I/day normalized the serum-free T3 level. A normalization of iodine metabolism is only given if both the iodine and selenium requirements are met.

The main excretory routes for iodine are the kidneys and the breast, which compete with the thyroid for plasma iodine. Urine and milk are good indicators of the iodine status and intake, if fecal iodine excretion of humans (about 10–80 μg/day) is taken into consideration. On average, omnivores and vegetarians excrete 81 to 85% of the consumed iodine renally and 15 to 19% fecally. Their apparent iodine absorption rate is 82 to 86%. After I supplementation of the mineral mixtures fed to animals and of salt for humans, iodine concentrations in cows' and breast milk increased from 10 to >100 μg/L.

Copper in neurologic and neurodegenerative diseases

14.A.1
Overview of the role of copper in neurologic and neurodegenerative diseases and potential treatment with tetrathiomolybdate

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In a number of neurodegenerative disease, which include Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, and prion disease, copper is intimately involved in binding to relevant proteins, aggregate formation, and perhaps in producing damaging oxidant stress. The role of copper in these diseases, to the extent known, will be reviewed. Of these diseases, only in AD has the cumulative evidence become convincing that excess copper is involved with initiation or progression of the disease. In the other talks of this symposium, some of that evidence in AD will be presented. These results indicate that therapy to reduce copper levels may be beneficial in AD, and we propose that tetrathiomolybdate (TM) is the ideal drug for such trials. TM's mechanism of action in the blood involves formation of a three-way complex with copper, albumin, and itself, which renders the relatively mobilizable, and potentially toxic, copper unavailable for cellular uptake. In this manner, TM detoxifies copper in neurologically presenting Wilson's disease and lowers the availability of copper in the body in other disease states. For example, by limiting copper availability, TM has been shown to be an effective therapy in a variety of animal models of fibrosis, inflammation, and autoimmunity. These include bleomycin induced pulmonary fibrosis, carbon tetrachloride induced cirrhosis, concanavalin A induced hepatitis, acetaminophen induced liver damage, doxorubicin induced heart damage, immune modulated bovine collagen induced arthritis, autoimmune diabetes in the non-obese diabetic mouse, autoimmune systemic lupus erythematosus, and EAE induced multiple sclerosis. Mechanisms of protection may involve inhibition of damaging cytokines and protection against oxidant damage. We postulate that TM will limit copper availability and toxicity in AD, and clinical trials are planned.

14.A.2
Copper, cholesterol, and cognition: animal and human evidence

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Increased circulating cholesterol levels are associated with serious medical concerns and represent a mounting problem as elevated cholesterol becomes more widespread. Besides an obvious increase in coronary artery disease and stroke, emerging data suggest an increased risk of cognitive impairment with increased circulating cholesterol. Furthermore, excess cholesterol promotes production of the Alzheimer's disease (AD) neurotoxin, amyloid beta (Ab). We identified a further link between copper, cholesterol, and cognition in the cholesterol-fed rabbit model of AD. It was shown that dietary cholesterol induces central production of Ab and that copper determines if overproduced Ab is cleared to the blood or accumulates in brain. Levels of copper ion were manipulated by adding trace amounts of the metal to an animal's distilled drinking water. Memory deficits (80%) were associated with brain Ab deposits in cholesterol-fed animals administered 0.12 PPM copper water, compared to cholesterol-fed animals drinking unaltered distilled water where there was minimal accumulation of Ab. Cholesterol and copper may independently influence copper levels in blood and brain. Adding copper to the water of animals fed normal chow increases blood and brain copper levels

compared to animals fed normal chow and drinking distilled water. Copper levels are increased in the blood and unchanged in the brain of cholesterol-fed animals drinking distilled water, while levels are decreased in brain and increased in the blood of cholesterol-fed animals drinking copper supplemented water. Copper may inactivate Ab, suggesting low brain copper could enhance Ab-induced degeneration in AD. We have previously reported elevated circulating copper and cholesterol levels in low-function controls (LFC), MCI and AD compared to high-function controls (HFC). We evaluated brain copper, zinc, and iron levels in HFC, LFC, MCI and AD, and Parkinson's (PD) patients with and without dementia.

14.A.3

Excess of serum 'free' copper in patients with Alzheimer's disease

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Current research hypotheses on AD point at oxidative stress and metal level imbalance as potential factors leading to the accumulation of ß-amyloid (Aß) in the brain. Copper—together with zinc and iron—accumulates into amyloid plaques, and it seems that copper-Aß interaction in AD can result in H_2O_2 generation, Aß aggregation and consequent tissue damage. Copper distribution and its interaction with Aß seem to be crucial in the AD brain, whose circulating copper also appears abnormal. In fact, we have shown that 'free' (i.e., not bound to ceruloplasmin) copper is elevated in the serum of AD patients and correlates with the patients' cognitive decline, with markers of AD and with copper in the CSF, with apolipoprotein E epsilon 4 allele, with the slowing of EEG rhythms, with abnormalities of liver function (Squitti et al., 2002, 2005, 2006, 2007) and it seems to be prognostic of cognitive decline. 'Free' copper is a 'low molecular weight' copper generally associated and exchanged between micronutrients and albumin. Its level is low in normalcy (<1.6 μmol/L), but it is crucial for the transport of copper from plasma to the brain. In particular, we have demonstrated that serum 'free' copper can filter through a membrane with a cut-off of 10kDa and, via a clearance measure, that about 3% of serum 'free' copper can cross the blood–brain barrier in living patients. However, the reliability of 'free' copper as a marker of AD is under discussion. Evidence in favour of 'free' copper in AD comes from two studies, a past one from Snaedal et al. (1998) and a recent one from Kessler et al. (2006). Snaedal et al. (1998) reported similar levels of absolute serum copper in AD and controls, but significantly lower concentrations of ceruloplasmin. This corresponds to higher concentrations of 'free' copper in AD patients than in controls. Similarly in a recent study by Kessler and colleagues (2006), a 'free' copper value of 4 μmol/L can be calculated in AD patients

14.A.4

Dietary fat and copper effects on cognition

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There is evidence from epidemiologic and animal studies to suggest that dietary fat composition and trace metals may be related to neurodegenerative diseases of the brain. These hypotheses were investigated in the Chicago Health and Aging Project, a 1993–2002 study of community residents aged 65 years and older that included the administration of four cognitive tests to all participants and clinical evaluations for incident Alzheimer's disease. Dietary assessment was by food frequency questionnaire. Daily nutrient intakes were computed from food and vitamin supplement sources. Fat composition was associated with incident Alzheimer's disease and with cognitive decline over 6 years. Saturated and trans fat intakes increased disease risk and cognitive decline, and $n-3$, $n-6$, and $n-9$ fatty acids were protectively associated. Dietary intakes of the trace metals, copper, zinc, and iron (food plus vitamin supplements) were not associated with cognitive decline overall. However, among persons who had high intakes of saturated and trans fats, high copper intake was associated with faster rate of cognitive decline over 6 years. The increase in the rate for the high-fat consumers whose total copper intake was in the top 20% (1.6 mg/day) was the equivalent of 19 years of

older age and was highly statistically significant (<0.0001). A similar but weaker association was observed for copper intake from food sources only. There was strong linear relation between copper dose in vitamin supplements and greater cognitive decline. By contrast, there was little evidence of association between cognitive decline and intakes of zinc and iron. These data suggest that high intake of dietary copper among persons who have a high-fat diet may be associated with accelerated loss in cognitive ability.

14.A.5

Investigations on the role of copper in human neurodegenerative diseases using model systems

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Copper plays a key role in the brain due to its requirement by several critical cuproenzymes. Cu is involved in several neurodegenerative diseases. Both Cu deficiency and Cu excess can lead to oxidative stress and severe disease. Alzheimer's disease (AD) is a severe neurodegenerative disease and is the most common form of dementia in the elderly. Senile plaques contain aggregates of beta amyloid (Abeta) derived from amyloid precursor protein (APP) and are found in the brain of AD patients. Neurofibrillary tangles due to aggregates of Tau protein also contribute to AD pathology. Cu is found in Abeta in plaques, and Abeta-Cu oligomers are toxic. Our data suggests that Cu can be effluxed from neurons bound to a cleaved product of APP, probably Abeta. We have also found that Cu regulates the APP promoter which is consistent with APP or an APP cleavage product being involved in Cu homeostasis. The excessive loss of Cu bound to Abeta can lead to a functional Cu deficiency in neurons which would result in oxidative stress and contribute to Alzheimer's pathology. This is supported by our findings of reduced CuZnSOD activity in N2a neuroblastoma cells expressing familial AD APP mutants. Hence, both an intracellular Cu deficiency and an extracellular Cu excess are likely to be major contributors to Alzheimer's pathology. Our data also shows that mutation of an APP ectodomain Cu binding site results in abnormal processing of APP and accumulation of APP in the membrane. To test the role of high and low Cu in AD, we are also using a *Drosophila* model where familial AD mutant APP or mutant Tau is overexpressed in strains which have been genetically engineered to have either high or low Cu by overexpressing either Cu uptake or Cu efflux transporters. The "rough eye" phenotype is being used as an index of neurological damage. Current data using this controlled system suggests that flies with high Cu levels showed an enhancement of neurodegeneration due to mutant Tau.

Health effects of low dose exposure to toxic metals

14.B.2

Proteomic and metabolomic biomarkers for assessing low dose toxic trace element interactions: an overview

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Toxic trace element-specific alterations in target cell protein expression patterns (proteomics) and changes in essential metabolic pathways (metabolomics) have proven extremely valuable for early detection of toxicity. These molecular approaches have been shown to be capable of detecting specific response patterns for both individual elements and mixtures of these elements as a function of dose, duration of exposure, and gender. As these pathways are highly conserved across species, it is possible to make some extrapolations from experimental model systems to humans. This presentation will provide an overview of studies designed to examine low-dose biomarker effects from combined exposures to lead, cadmium, and arsenic using an in vivo model system. In addition, data from similar in vivo and in vitro studies will examine how combined proteomic and metabolomic biomarker approaches have provided useful information on early compound and gender-specific responses from exposures to the semiconductors gallium arsenide and indium arsenide. An overall conclusion from these studies is that translation of

findings from modern basic scientific approaches can greatly improve risk assessments for toxic trace elements and help to delineate populations at special risk within the general population.

14.B.3

Neurological and renal effects of low dose exposure to elemental mercury exposure from amalgam in children

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Dental amalgam constitutes the most common source of exposure to elemental mercury in the general population, and the possible health risks have been debated for a long time. Many studies have been performed in adults exposed to low levels of elemental mercury, but until recently, a few data were available on this topic in children, who might be more vulnerable. In 2006, two large randomized controlled trials were published, comparing neuropsychological (full scale IQ, memory, attention, visuomotor tests) and renal outcomes (e.g., albumin excretion) between children treated with dental amalgam or composites over a 5-year trial period. The studies included more than 1,000 children from northeast USA or Portugal. These studies failed to demonstrate any statistically significant differences in adverse effects between treatment groups. The increase in urinary mercury in the amalgam-treated groups compared to controls treated with composites was, however, limited, on average about 0.5–1 μg/g creatinine. The results of these two trials will be reviewed, together with subsequent studies using this rich data set. The strengths and limitations of the two trials will be discussed as well as children's exposure to inorganic mercury in general and the results of some recent observational studies of possible adverse effects.

14.B.4

Manganese exposure as a determinant of parkinsonian damage

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Manganese is an essential element for humans and animals and plays an important role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans. Homeostatic mechanisms regulate the absorption and excretion rates to keep manganese concentration within a strict range. Nevertheless, short-term exposure to high doses or prolonged exposure to low doses can determine a manganese overload in the central nervous system, given the slow elimination rate from this organ. In cases of overload, manganese accumulates in the globus pallidus of the basal ganglia, where it can cause cellular damage on the GABAergic and dopaminergic pathways. As a consequence, motor function and coordination of fine movements are affected, and mood regulation as well with marked aggressivity. Human exposure to air concentration higher then 1 mg/m^3 can determine the clinical picture of manganism, an atypical Parkinsonism that shows clinical differences from the typical features of Parkinson's Disease. After prolonged exposure at much lower levels, manganese may also act as an environmental trigger and favor the onset of typical Parkinsonian disturbances. This can be determined by a damage of the dopaminergic neurons of the substantia nigra-pars compacta, which is located very closely to the globus pallidus and shares various interconnections within the basal ganglia. This hypothesis is presented based on experimental animal studies and human epidemiological studies on exposed workers and the general population resident in the proximities of polluting sources.

14.B.5

Kidney and bone effects of low-dose cadmium exposure in Sweden

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Cadmium is a toxic environmental pollutant that all are exposed to via foods considered healthy, such as whole grains, vegetables, and potatoes. High-cadmium exposure is well-known to cause kidney and bone damage, but these associations at low-level cadmium exposure need further attention, and the critical exposure level in the general population needs to be defined. We investigated health effects of cadmium exposure

among 820 women, aged 53 to 64 years, within a population-based women's health survey in southern Sweden (WHILA; 71% response rate). The area has no known historical cadmium contamination. Specifically, we assessed the associations between cadmium exposure and tubular and glomerular function, forearm bone mineral density, and markers of bone metabolism. We also calculated the benchmark dose, assessed serum 1,25 dihydroxy vitamin D3 and fracture incidence. Associations were evaluated in multiple linear regression analysis including information on the possible confounders or effect modifiers. The exposure levels were generally low; the median urinary cadmium was 0.52 μg/l adjusted to the mean urinary density (1.015 g/ml), corresponding to 0.67 μg/g creatinine, and blood cadmium was 0.38 μg/L. Our results showed associations related to negative effects of low level cadmium exposure on tubuli, glomeruli, and bone. Cadmium seemed to potentiate diabetes-induced effects on kidney and postmenopausal bone resorption. The obtained benchmark doses (95% lower confidence bounds; BMDL) of urinary cadmium were lower (0.4–0.5 μg/L at 5% additional risk) than the critical concentrations previously reported. The BMDL for glomerular effects was only slightly higher (0.55 μg/L) than that for tubular effects. Although these effects were small, they should be considered as early signals of potentially more adverse health effects.

14.B.6

Factors influencing dose–response relationships of cadmium in humans—diabetes, metallothionein, and metallothionein antibodies

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Cadmium, an environmental pollutant, causes a number of adverse health effects, particularly toward the kidneys. In this study, we focus on the relationships between MT-Ab, urinary cadmium (UCd), and kidney function, in a Chinese diabetic 2-microglobulinβ population. The results show that tubular biomarker, urinary (UB2M) increased significantly after the elevation of MT-Ab and urinary cadmium; however, glomerular biomarker urinary albumin (UALB) did not display such a pattern. Those patients with tubular but not glomerular damage have highest UB2M, MT-Ab and UCd levels compared with those subjects without renal dysfunction and those with glomerular but not tubular dysfunction. In both gender subjects, UB2M statistically correlate with UCd (r=0.25 and 0.24, $p<0.05$) and MT-Ab (r=0.21 and 0.21, $p<0.05$), while UCd did not show any significant correlation with MT-Ab ($p>0.05$). After adjusting for potential confounding covariates, logistic regression, only fasting capillary blood glucose(FCG) had a significant effect on glomerular dysfunction, and the risk increased 2.75 times (95%CI: 1.12–6.74) when FCG was higher than 6.1 mmol/L. However, MT-Ab levels, UCd levels, and smoking habits significantly increase the risk to develop tubular dysfunction within this diabetic population. The odds ratios (OR) for MT-Ab levels, UCd levels, and current smoking were 5.56 (95% CI: 2.25–13.73), 3.34 (95% CI: 1.17–9.53), and 3.51 (95% CI: 1.14–10.80), respectively. This study proved MT-Ab could potentate tubular dysfunction among diabetic subjects, and those patients with high levels MT-Ab were more prone to develop tubular damage.

Environmental stress and mineral homeostasis

14.C.1

Overview of human physiological responses to environmental extremes

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Millions of people live, work, and play in regions of the world where the weather is intemperate, or the elevation is sufficiently high that ambient oxygen pressure is lower than at sea level. In addition, while relatively few people have traveled in space up to now, increasing numbers of people may engage in space flight as the century proceeds. The environmental stressors of greatest physiological importance are hypoxia, cold, heat, and gravity. When humans are exposed to extremes of these environmental stressors, a variety of physiological responses manifest that assist in reestablishing and/or maintaining homeostasis. Those physiological responses could have implications for dietary micronutrient requirements, e.g., sweating-associated trace element losses. Furthermore, physiological responses to exercise can be a major factor influencing nutritional

requirements, and responses to strenuous physical activity can be exacerbated by extreme environmental conditions. Therefore, to begin considering how extreme environments affect dietary trace element requirements, this presentation will identify key environmental factors constituting biologically significant stress for humans. Furthermore, the physiological adjustments elicited by those stressors, especially those exhibited by physically active persons engaging in strenuous activities, will be discussed.

14.C.2
Iron homeostasis in environmental extremes

James McClung
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Dietary iron confers biological function through incorporation into a series of proteins, including hemoglobin and myoglobin for the transport and storage of oxygen and cytochromes for the oxidative production of cellular energy. Iron deficiency is the most prevalent micronutrient deficiency disorder in the world, affecting mainly premenopausal women. Changes in immune function, cognitive performance, and work capacity have been described in humans and animals with suboptimal iron status. Iron status indicators may be affected by a number of environmental conditions, including altitude and exposure to heat. Furthermore, physical activity seems to affect iron status. A recent line of studies has investigated the effect of combat training on iron status in female military personnel. Decrements in iron status indicators, including serum ferritin, transferrin saturation, and red cell width have been reported after 8 weeks of military training. This review will focus on the effects of extreme environmental conditions on iron homeostasis, citing recent studies with female military personnel as an example of a population that may be sensitive to decrements in iron status. Furthermore, potential nutritional interventions to mitigate the effect of environmental extremes on iron status will be discussed.

14.C.3
Mineral homeostasis at high altitude: focus on antioxidants

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Accelerated metabolic rate and free radical production at high altitude increase macronutrient and vitamin requirements; however, data concerning mineral homeostasis at this extreme environment are scarce (Butterfield, 1999). As minerals are important structural components of many antioxidant enzymes, measurements of specific enzyme activities and oxidative stress can yield insight into mineral status during exposures to high altitude. Particularly, the Cu, Zn isoform of superoxide dismutase (SOD1) and Se-dependent cellular glutathione peroxidase (GPx) have been studied during both short-term (<24 h) and prolonged (>24 h) exposures to high altitude. In general, antioxidant capacity has been sufficient to counterbalance oxidative stress during short-term exposures. Specifically, acute altitude exposure did not increase whole-body markers of oxidative stress or antioxidant activity of erythrocyte SOD1 or GPx in individuals eating a well-balanced diet, although repeated short-term exposures tended to deplete antioxidant reserves over time (Subudhi et al., 2001). Prolonged altitude exposure has been associated with increased markers of oxidative stress, but has had equivocal effects on antioxidant enzyme systems. Se status was not compromised during the first 14 days at high altitude, as GPx activity was similar to low altitude conditions and unaffected by Se supplementation (Subudhi et al., 2004). In addition, SOD1 activity was increased over 13 months at high altitude, suggesting favorable Cu and Zn status (Rawal et al., 1999). To date, research does not indicate a significant disruption in mineral balance at high altitude, yet future studies are needed to determine the effect of altitude on actual plasma and tissue concentrations of various minerals.

14.C.4
Mineral homeostasis during spaceflight: bone demineralization

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Bone loss during spaceflight remains a significant challenge to astronaut health on space exploration missions. Associated short-term risks, including renal stone formation, and long-term risks of fractures and increased skeletal fragility, are all matters of concern. Spaceflight-induced bone loss is one of the effects of

weightlessness in which nutrition clearly can play a role, either positive or negative. Findings from spaceflight and studies using ground-based analogs of flight (such as bed rest) clearly indicate that bone resorption increases during and after flight. Maintaining vitamin D status remains a challenge for long-duration space travelers, who lack exposure to ultraviolet light in their shielded craft. A great many nutrients besides calcium and vitamin D play a role in bone health and may play a role in astronaut health during spaceflight. These include excess dietary sodium, excess dietary protein, and insufficient vitamin K. Excess dietary protein and sodium both seem to affect acid/base balance, and subsequently to increase bone resorption. This phenomenon appears to be exacerbated during bed rest. This provides one example of how studying the relationship between nutrition and physiology in spaceflight analogs has proven valuable to space travelers and has also furthered our understanding of health and disease on Earth. Nutrition—defined as the appropriate nutrient intake and balance of nutrients—is essential for crew health on space exploration missions. Exercise and/or pharmaceutical countermeasures will not mitigate weightlessness-induced bone loss if the nutrients required for bone health are not available (or are available in excess). Much work remains to clearly define the nutritional requirements for space travel and whether dietary alterations may provide safe and effective countermeasures.

14.C.5
Mineral losses during extreme environmental conditions

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Minerals are nutrients that are conserved by the body. During exposure to environmental stimuli, such as heat and/or exercise, the excretion of minerals, macro- (Na, K, Ca, Mg) and micro- (Cu, Fe, Zn), occurs through the body surface in the form of cellular desquamation and sweat, as well as in the urine. Whereas it is well established that urinary excretion of minerals increases after physical activity, compared to no activity, there is uncertainty regarding surface mineral losses during acute, compared to prolonged bouts of exercise and on repeated days in a hot environment. Surface losses of minerals, total of cellular and sweat, have been sampled from various sites of the body including upper and lower arm, chest, back, thigh, and forehead by using different collection devices or materials. These losses are expressed as concentrations of minerals (mg/L or ng/L), are appreciable, and show wide intra- and interindividual variability. In general, conservation of minerals occurs during prolonged and repeated bouts of exercise in the heat. Methods of sweat collection, sources of contamination, differences by sex, and trends of mineral losses in heat-acclimated and heat-unacclimated individuals will be presented. The impact of surface losses of minerals on body mineral retention also will be discussed.

Molecular mechanisms of metal induced disease

14.D.1
Changes in dietary iron levels affect brain manganese accumulation and distribution

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Occupational exposure to manganese (Mn) has been associated with the onset of psychological and motor symptoms in some individuals, leading to a phenotype called manganism. As Mn and Fe are transported by the same proteins, we determined whether changes in dietary Fe levels alter the brain Mn deposition patterns. Previous data from magnetic resonance imaging (MRI) and atomic absorption (AA) spectroscopic techniques demonstrated regionally specific and later more global brain Mn accumulation in treated populations compared to the controls. In the current study, two groups of adult male rats were fed either Fe-deficient (3 mg Fe/kg) or Fe-supplemented (30 mg Fe/kg) chow for 14 weeks. Both groups were also given weekly intravenous injections of 3 mg Mn/kg. Animals were weighed weekly, a measure of general health. At the conclusion of the study, brains were removed and dissected into various regions and the amount of Mn and Fe determined. Data was compared to the control and Mn-treated animals from a previous study. Fe-supplemented animals weighed significantly less than other groups throughout the

course of the study (one-way repeated measure ANOVA, $p<0.05$). Atomic absorption spectroscopy indicated no difference in brain Mn or Fe levels between the Fe-deficient or Fe-supplemented groups. However, both groups showed an increase in brain Mn levels in the cerebellum, brain stem, striatum, and cortex compared to the control and Mn-treated cohorts ($p<0.05$). Interestingly, there was a trend to decreased brain Fe levels in both Fe-modulated groups. This reached statistical significance in the Fe-deficient populations in the cerebellum, brain stem, midbrain, and cortex ($p<0.05$) compared to the control. These data suggest that any change in dietary Fe levels exacerbates brain Mn accumulation and alters normal brain Fe distribution in multiple discrete brain regions. This work was supported by DoD W81XWH-05-1-0239 project 04149002 (MA and VF) and NIEHS ES 10563 (MA).

14.D.2

Molecular carcinogenicity of nickel ions targets HIF-1 prolyl hydroxylases and histone-3 lysine 9 demethylases

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Both soluble and insoluble nickel compounds have been implicated as carcinogens. Ni ions compete with Fe transport and are taken up into cells in place of Fe via the divalent metal ion transporter, resulting in a depletion of cellular Fe. HIF-1 alpha transcription factor is stabilized and then activates transcription of its numerous target genes (VEGF, glucose transporters, glycolytic enzymes, etc.). The primary mechanism by which Ni ions stabilize and persistently activate HIF-1 alpha is by inhibition of Fe, oxoglutarate, ascorbic acid-dependent dioxygenases that regulate the stability and transcriptional activity of HIF-1 alpha (PHD1-3 and FIH). Ni ions are believed to displace and deplete Fe from these enzymes. Hypoxia and Ni ion exposure result in a global loss of histone acetylation and an increase in the methylation of H3K9 resulting in a loss of DHFR and Mlh1 (mismatch repair) expression in nucleosomes having H3K9 dimethylation as determined by the CHIP assay. Recent experiments have demonstrated the existence of a H3K9 demethylase that shares similarity to the Fe, oxoglutarate, and ascorbic acid-dependent dioxygenases. We hypothesized that Ni ions inhibited these demethylases causing enhanced global H3K9 methylation, which will lead to DNA methylation and gene silencing. We have expressed recombinant JHDM2A (H3K9-demethylase), and the 50% inhibitory level for Ni ions is 25 μM. In contrast, purified aconitase is not significantly inhibited at 10 mM Ni ions in vitro. Ni ions favor the binding of the JMJC Fe-containing domain of these dioxygenase enzymes, and we hypothesize that all of these enzymes represent major targets for Ni ions in the cell. In particular, the stabilization of HIF-1 alpha and histone methylation contribute to Ni ion carcinogenicity.

14.D.3

Reprogramming of histone marks by chromium

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Hexavalent chromium is among the top 20 in the priority list of hazardous substances and is commonly found as an environmental co-contaminant with polycyclic aromatic hydrocarbons, such as benzo[a] pyrene (B[a]P). Exposure to binary mixtures of these two agents causes multiple adverse health effects that do not show additive or synergistic associations. As an occupational hazard, more than one million workers in the USA alone are exposed to chromium (VI) in the work place. Chromium exposure alters inducible gene expression, forms chromium-DNA adducts, and chromium-DNA cross-links, and disrupts transcriptional activator/coactivator complexes. In addition, chromium has been associated with the incidence of birth defects and congenital malformations. We find that chromium blocks B(a)P-inducible gene expression by interfering with the assembly of productive transcriptional complexes at the promoters of over 50 different Ah receptor (AHR)-inducible genes involved in a variety of signal transduction pathways. These effects of chromium result from the inhibition of critical chromatin remodeling steps necessary for gene transactivation, including release of complexes of DNA methyl transferase-1 (DNMT-1) and histone deacetylase-1 (HDAC1) from the promoters of affected genes and subsequent recruitment of p300. Chromium-induced repression is inhibited by 5-aza-deoxycytidine. Chromatin immunoprecipitation assays show that Cr exposure leads to an inhibition

of critical histone marks connected with B(a)P-mediated induction of gene expression, such as histone H3 S10 phosphorylation, H3 K4 tri-methylation, and both H3 and H4 acetylation. These data help develop an understanding of the molecular mechanisms underlying the toxicity associated with environmental chromium exposures and provide an experimental approach to the analysis of epigenetic modifications induced by chromium (supported by NIEHS grants P30 ES06096, 2R01 ES06273, and 1R01 ES10807).

14.D.4

Arsenic cardiomyopathy and molecular mechanism

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Arsenic trioxide is highly effective in the treatment of acute promyelocytic leukemia (APL). In September 2000, the Trisenox brand of arsenic trioxide for the treatment of relapsed and refractory APL was approved in the United States. A clinical report has shown a serious ventricular tachycardia at the therapeutic doses of arsenic trioxide in APL patients. To investigate the cardiotoxic effect of arsenic trioxide, a mouse model of arsenic cardiotoxicity was used. Mice were injected intraperitoneally with arsenic trioxide in 5 mg/kg/d for 30 days, a dose regimen that has been shown to produce plasma concentrations of arsenic within the range of those present in arsenic-treated APL patients. Analysis of myocardial function revealed that arsenic caused a significant decrease in the maximum rate of rise in intraventricular pressure during ventricular contraction (MAX dP/dt) and significant increases in the end diastolic pressure and ventricle minimum diastolic pressure. In response to b-adrenergic stimulation the arsenic-treated heart did not show increase in MAX dP/dt, which was observed in the saline-treated controls. The functional alterations were accompanied by cardiomyopathy as revealed by histopathological and ultrastructural examination. Furthermore, arsenic caused myocardial apoptosis, as determined by a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay, which was confirmed by caspase-3 activation detected by enzymatic assay. The arsenic cardiotoxic effects can be prevented if metallothionein, a thiol-rich small protein, is elevated in the heart of transgenic mice. Our study thus demonstrates that arsenic, in a dose that could produce clinically comparable serum concentrations to those observed in humans, causes cardiotoxicity. The arsenic cardiac toxicity is related to its interaction with intracellular thiols. Therefore, elevation of small thiol-protein, metallothionein, greatly attenuated arsenic cardiotoxicity.

Advances in analytical detection of trace elements in biological tissues

14.E.1

Ion and electron beam methods for biological trace element characterisation at bioceramic/cell interface

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Ion and electron beam methods of analysis are based on X-ray fluorescence induced after interactions between charged particles and atoms present in the samples. They permit to obtain simultaneously multielemental maps and concentrations measurements of major, minor, and trace elements with a spatial resolution from micrometer to nanometer. Micro-PIXE, STEM associated to EDXS are useful techniques to determine locally concentration gradients at biomaterial/cell interface. The prerequisite for bioceramics (calcium phosphates, bioactive glasses) to bond to living bone is the formation of biologically active apatites on their surface in the body. Reactions and bioactivity mechanisms between bioceramics and cells depend on the material composition in major and trace elements. Knowledge of the local concentration and species of ions released at the ceramic/biological fluid/cell interface is primordial to understand the complex interfacial reactions and physicochemical properties of ceramics. This work characterises the interface that bioactive ceramic particles develop after interactions with biological fluids with or without cells. Results demonstrate material dissolution, ionic leaching, and precipitation of a biomimetic apatite layer at bioceramic surface. This bioactive layer contains biological relevant trace elements. Cell viability can be altered by the presence of bioactive ceramics particles and their features. Determination of intracellular ionic concentrations of cells in a state near the physiological state is of importance to better study cell viability. Use of cryo-methods for cell fixation limit

diffusion phenomenon of ions and preserve the chemical identity of specimens. Then, X-ray analysis of samples allows to study intracellular ions like Na, K, Cl. Moreover, it is also possible to evaluate the intracellular concentration of Mg, P, and S at the subcellular level. Thus, the K/Na ratio can be used as a sensitive criteria of cell viability.

14.E.2

X-ray fluorescence technique on trace element determination in biological samples

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Elemental composition of tissues and, in particular, trace elements has gained interest over the last few years, due to the increasing knowledge of the role of some elements and the correlation between abnormal concentration of some elements and pathological effects. Trace elements, although in concentrations of the order of g g^{-1}, play an important role in the organism. Some trace elements are "essential elements" for the organism, which means that the organism can neither grow nor complete its life cycle if the element is not supplied in sufficient quantity. Deficiency or excess of the element leads to specific diseases. On the other hand, between the trace elements are the "toxic elements", mostly the heavy metals. The toxic effect of the heavy metals is at different levels and can depend on the chemical form of the element. Essential elements can also become toxic at high concentration, and there is a wide range of toxicity, depending on the element and the preference tissue for accumulation. Studies of toxic elements, e.g., cadmium and its effects in plant cells, Hg in dolphins tissues, Pb elemental profiles in human teeth collected from contemporary miners, fishermen, and patients with renal insufficiency and populations of different pre-historical periods will be presented. The used technique for these studies is X-ray fluorescence spectrometry. It is based on the ionization of the atoms of the sample by means of an incident X-Ray beam. The atoms getting back to its fundamental state emit characteristic radiation. The detection of these photons allows identification and quantification of the elements present in the sample. Quantitative calculations for S, Cl, K, Ca, Mn, Fe, Co, Cu, Zn, As, Sr, Ba, and Pb in liver, brain, and kidney of healthy and cirrhosis disease human samples were performed.

14.E.3

Alternative testing methods in food nanoparticles toxicology research for a safe food nanotechnology development

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The new EU Chemical Policy foresees regulatory actions to prevent health risks of exposure to chemicals. Different kinds of toxicity data must be produced to improve the scientific basis of risk assessment. In this context, ECVAM has been set up to coordinate, at European level, activities to promote the scientific and regulatory acceptance of alternative toxicity testing. In particular, advanced approaches that have less or no dependence on animal experimentation (cell cultures) represent the basis of "in house" projects concerning development and validation of mechanistically based in vitro toxicity testing. In this context, such approaches have a great potential in the emerging area of toxicology research on nanomaterials. In particular, the possibility that food is designed by shaping molecules and atoms at an extremely small scale is opening up a whole universe of new applications. Unfortunately, in spite of the great excitement about the potential benefits offered by nanotechnology in food industry, a huge health and safety questions remain unsolved. In particular, there is a gap in the current regulation of nanomaterials, and there are emerging toxicological evidences to fear that manufactured nanomaterials could have undesirable health effects. In this context, food nanotoxicology research is an urgent need for the assessment of the health impact of nanomaterials relevant to food industry and food processing, and it is important that the regulations are tightened up so that nanomaterials are assessed, both in terms of testing and labeling as new chemicals. The aim of this work is to review and to show the potential that in vitro cellular and noncellular assays can have in food nanotoxicology research to take into account the substantial ethical, political, financial, and regulatory pressures to reduce, refine, and replace animal use with alternative nonanimal assays (3Rs approach).

Mineral elements and molecular signaling as it relates to human disease

14.F.1
Role of zinc in endothelial cell function: implications in atherosclerosis

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Zinc has multiple roles in maintaining the physiological conditions of the cardiovasculature. We hypothesized that a depressed zinc status may be involved in either initiation of vascular endothelial cell injury or inadequate vascular tissue repair. Thus, zinc requirements of the endothelium may be increased during inflammatory conditions that exist in cardiovascular disease. To assess the protective mechanisms of zinc during an endothelial cell (EC) inflammatory and zinc, response, EC were activated by treatment with linoleic acid, or TNF deficiency was induced by treatment with the membrane permeable zinc chelator TPEN. We demonstrated that zinc deficiency induced oxidative stress, increased the DNA binding activity of NF-kB and AP-1, and increased EC production of IL-6. Peroxisome-proliferator-activated receptors (PPARs) may have antiatherogenic properties. We found that zinc deficiency decreased PPAR activation and protein expression. In contrast, zinc supplementation markedly increased PPAR activation and expression, which was correlated with downregulation of DNA binding activity of NF-kB and AP-1. Our data demonstrate that zinc exhibits potent antioxidant and anti-inflammatory properties. Furthermore, zinc appears to protect against EC activation and inflammation by functioning as a critical component of the PPAR transcription factor complex. A recently completed in vivo study suggests that in an atherosclerotic mouse model, PPAR signaling is compromised during zinc deficiency and that adequate dietary zinc is critical for proper function of the antidiabetic medicine rosiglitazone. In summary, our data suggest that zinc can downregulate the pathways of signal transduction leading to an inflammatory response and to disruption of endothelial cell integrity. Thus, zinc may have critical nutritive and therapeutic roles in inflammatory diseases such as atherosclerosis (supported in part by grants from NIEHS/NIH (ES 07380), USDA/NRI (2001-01054), and UK AES).

14.F.2
Oxidative stress in tumor progression: intervention by selenium

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Myofibroblasts, pivotal for tumor progression, populate the microecosystem of reactive stroma. The potential involvement of reactive oxygen species in the mesenchymal–mesenchymal transition (MMT) of human dermal (stromal) fibroblasts to myofibroblasts was studied. We addressed the question of whether intervention with micronutrients such as selenium and antioxidants may affect this process in stromal cells and the invasive capacity of skin-derived squamous carcinoma cells. Using a broad spectrum of biochemical and molecular biological methods [1], we found that upon treatment with conditioned medium of tumor cells and transforming growth factor beta 1 (TGFβ1), respectively, the intracellular level of reactive oxygen species (ROS) of fibroblasts was increased via a protein kinase C (PKC)-dependent pathway. The elevated ROS level, assessed as lipid hydroperoxides, initiates a signaling process resulting in both MMT and release of proinvasive signals. Using a filter-based in vitro invasion assay, a significant increase in invasive capacity of tumor cells was measured, based on a paracrine effect of myofibroblast-derived hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6). Preincubation of fibroblasts with selenite, selenoprotein P, or other antioxidants lowered TGFβ1-initiated lipid peroxidation, subsequently resulting in inhibition of expression of alpha-smooth muscle actin (αSMA), a biomarker for myofibroblastic cells, in lowering of the invasive capacity of tumor cells. Taken together, TGFβ1 is considered to play a major role in the generation of "tumor-educated" fibroblasts, namely, myofibroblasts. The novel concept of stromal therapy, meaning the protection of stromal cells against the dominating influence of tumor cells in tumor–stroma interaction by micronutrients and/or antioxidants, may contribute to anti-invasive and anti-metastatic strategies.

14.F.3
Effect of zinc on DNA integrity and cancer risk

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There is increasing evidence that micronutrient deficiencies may damage DNA and increase cancer risk. A large proportion of the population has inadequate zinc intakes. Zinc is a component of over 300 proteins, including DNA-binding proteins with zinc fingers, Cu/Zn superoxide dismutase and DNA repair proteins such as p53, a zinc protein which is mutated in half of human tumors. It can be hypothesized that insufficient zinc intake can impair antioxidant defenses and compromise DNA repair mechanisms, making the cell highly susceptible to oxidative DNA damage. Using of both genomic strategies (using microarray technology) and functional assays, we have found that zinc deficiency increases DNA damage, oxidative stress, expression of DNA repair enzymes but impairs downstream signaling events in a cell culture model. This data demonstrates that zinc deficiency causes oxidative stress and DNA damage, but important downstream signals leading to proper DNA repair are lost without zinc. In vivo, we have also found that zinc deficiency increases DNA damage in peripheral blood cells and increases several markers of oxidative stress. Consequently, zinc deficiency not only causes oxidative stress and induces DNA damage but also compromises the cell's ability to repair this damage. Zinc deficiency also significantly increases the sensitivity of cells to DNA-damaging agents, such as low dose ionizing radiation. Moreover, expression profiles in zinc-adequate and zinc-deficient cells after irradiation differed markedly, suggesting alterations in transcriptional response with zinc deficiency after exposure to DNA damaging agents. This work strongly suggests that zinc deficiency has a detrimental effect on DNA integrity and emphasizes the importance of good nutrition in the prevention of cancer.

14.F.4
Maintaining copper balance: roles of copper transporters and chaperones

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Copper (Cu) is an essential micronutrient that plays an important role as a catalytic cofactor for a number of metalloenzymes involved in various biochemical processes including cellular respiration, antioxidant defence, development of connective tissue, pigment biosynthesis, iron homeostasis, and neurotransmitter production. Cu's redox chemistry, however, also makes it a potentially toxic metal if not properly utilized. Therefore, cells have evolved elaborate systems to control the uptake, distribution, and elimination of Cu. Characterization of Cu transporters that mediate Cu uptake or efflux and the identification of Cu chaperones that deliver Cu to specific cellular targets have greatly improved our understanding of the mechanisms by which cells effectively use Cu and maintain Cu homeostasis. In yeast, Cu transporter 2 (Ctr2) is thought to function as a vacuolar Cu transporter that mobilizes Cu from the vacuole to the cytosol. Cu chaperone for Cu/Zn superoxide dismutase (CCS) inserts Cu into the antioxidant enzyme Cu/Zn superoxide dismutase. Using a transient overexpression approach in COS-7 (monkey kidney) cells, we have shown that although the majority of Ctr2 is localized to the outer membrane of cytoplasmic vesicles, a small proportion is present at the plasma membrane where it promotes specific, saturable Cu uptake. We have also demonstrated that CCS protein is upregulated by Cu deficiency and that Cu regulates CCS degradation by the 26 S proteosome. Using a rat model, CCS was determined to be a sensitive indicator of mild Cu deficiency induced by moderately high intakes of zinc. The potential roles of Ctr2 in Cu transport in the context of known Cu-trafficking pathways in mammalian cells and the application of CCS as a biomarker of Cu status will be discussed.

Is copper involved in carcinogenesis/carcinostatics?

14.G.1
Possible association of Wilson's disease with hepatocellular carcinoma

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Wilson's disease (WD) is a hereditary disorder of copper accumulation that results in abnormally high levels of copper in the body, primarily in the liver. Hepatic copper accumulation can lead to chronic active hepatitis, cirrhosis, and fulminant hepatic failure. Although WD causes liver cirrhosis, hepatocellular carcinoma (HCC) is rare in patients with WD, compared to that of hepatitis C virus-related cirrhotic patients. However, a Long–Evans rat with cinnamon-like coat color (LEC) rat, an animal model for this disease, is known to exhibit HCC, suggesting that copper accumulation in the liver may be associated with carcinoma. There is a low incidence of HCC in patients with WD, but this may be attributable to the significantly shortened life expectancy in untreated patients. The effect of elevated hepatic copper levels on oncogenesis is still unclear. Our studies indicate that the accumulation of copper is not homogeneous in the liver of patients with WD, but is rather 1.4- to 3.6-fold higher in the right lobe than the left lobe. To examine the association between hepatic copper load and the development of carcinoma, we examined the localization of HCC in patients with previously reported WD. Twenty-three patients with WD-associated HCC were reported from 1959 to 2007. All of the patients had hepatic cirrhosis. The mean ages at diagnosis of WD and HCC were 33.8 years (6 to 66) and 37.9 years (12 to 66), respectively. Serological tests for anti-HCV antibody, HCV RNA, or both, were described in 5 of the 23 patients and were negative in all cases. Tests for HBV were performed in 13 of the 23 patients, and only one patient was positive for anti-HBs and anti-HBc antibody. From the reports with description of the position of HCC, HCC was found in the right hepatic lobe in 10 of 15 patients and in the left lobe in 4 of 15 patients. From these results, we will discuss the association of the accumulation of copper in the liver with carcinogenesis.

14.G.2

Carcinogenesis in LEC rat and patients with Wilson disease

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Wilson disease is an autosomal recessive disorder resulting from the defective functioning of copper transport P-type ATPase (ATP7B). It reveals a reduction in the rate of copper incorporation into ceruloplasmin and a reduction in the biliary excretion of copper. Copper accumulates in the liver, brain, cornea, kidney, and others. The major clinical signs and symptoms of the disease are cirrhosis, extrapyramidal signs and Kayser–Fleischer rings. The typical biochemical features of these patients are low serum ceruloplasmin levels and high urinary copper excretion. The hepatic copper content increases remarkably. Prognosis of Wilson disease is very poor without treatment. However, after the introduction of copper-chelating agents, the patient's prognosis improves dramatically. The Long–Evans cinnamon (LEC) rats, which are a mutant inbred strain isolated from Long–Evans rats, develop hepatitis and hepatocellular carcinoma in an autosomal recessive pattern of inheritance. Approximately 40% of LEC rats die of fulminant hepatitis, and the remaining 60% of LEC rats survive and develop hepatocellular carcinoma. They also demonstrate many clinical and biochemical features of Wilson disease, including a low serum ceruloplasmin level, a high urinary copper excretion, and remarkably increased copper content in the liver. A partial deletion at the 3′ end of atp7b, the rat gene homologous to ATP7B, has been identified in the LEC rats. The LEC rats are a rodent model for Wilson disease. Although human hepatocellular carcinoma is a well-recognized complication of cirrhosis, a relationship has not been recognized between hepatocellular carcinoma and Wilson disease. Authors expect that the prevalence of liver cancer in Wilson disease may increase as survival improves. The LEC rats will serve as an important model of investigation about the mechanism and prevention for this problem.

14.G.3

The effects of hepatic copper overload on development of hepatocellular carcinoma (HCC) in HCV-positive patients with chronic liver disease

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Although hepatitis C virus (HCV) is a major etiologic factor in hepatocellular carcinoma (HCC) in Japan and some European countries, the mechanism leading to HCC has not yet been elucidated. We have reported

that (1) Hepatic Cu content increases with progression of hepatic fibrosis but not hepatic Fe and Zn contents. (2) Accumulation of Cu in HCC, especially in small tumors (<40 mm), is greater than that in the surrounding liver parenchyma. (3) Cu exists in the form of Cu-metallothionein (Cu-MT) in HCC, and this Cu-MT produces hydroxyl radicals when H_2O_2 is produced, according to a study of Long–Evans rats with a cinnamon-like coat color which develop HCC spontaneously. We have suggested that Cu accumulation in the liver parenchyma may be closely related to hepatocarcinogenesis. On the basis of these results, we investigated the relationship between trace metals and the prevalence of HCC in patients with chronic hepatitis or hepatic cirrhosis caused by HCV. We measured the contents of Cu, Fe, and Zn in HCC tissue (n=112), dysplastic nodules (n=7), and liver parenchyma in patients with (n=112) and without (n=12) HCC. Metals were quantified in thin-needle biopsy specimens using the particle-induced X-ray emission method (PIXE). Cu level in liver parenchyma was higher in patients with HCC than in those without HCC (p<0.01), while there was no such difference in hepatic Fe. In patients with hepatic cirrhosis, Cu content in the liver parenchyma was higher in the presence of HCC than in its absence (p< 0.05). Multiple regression analysis showed that the only factor significantly associated with the coexistence of HCC in HCV-positive patients with chronic liver disease was the Cu level in the liver parenchyma. Hepatic copper overload may contribute to the development of HCC in HCV-positive patients with chronic hepatitis or cirrhosis.

14.G.4
Copper transporters regulate the cellular pharmacology of cisplatin

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There is now a large body of evidence to indicate that the copper (Cu) transporters CTR1, ATP7A, and ATP7B regulate the cellular pharmacology and cytotoxicity of cisplatin (DDP) and that these proteins can mediate acquired DDP resistance. Cells that have acquired resistance to cisplatin (DDP) demonstrate cross-resistance to copper (Cu) and vice versa. The cross-resistance between DDP and Cu is characterized by parallel changes in Cu and DDP accumulation and altered expression of the Cu efflux transporters ATP7A and ATP7B. Yeast, mouse, and human cells engineered to alter the expression of CTR1, ATP7A, or ATP7B exhibit altered sensitivity to both Cu and DDP. Detailed studies of uptake and efflux indicate that each protein individually can alter the cellular pharmacology of DDP, and in some cases, the DDP analogs carboplatin and oxaliplatin. The major Cu influx transporter CTR1 appears to be the primary transporter mediating the initial influx of DDP. Knockout of CTR1 in either yeast or mammalian cells markedly reduces their ability to accumulate either Cu or DDP. Recent studies have demonstrated that DDP triggers the internalization and degradation of CTR1 via a process that involves macropinocytosis and that DDP is much more potent than Cu in down-regulating CTR1 expression. While other transporters may also participate in the influx and efflux of the platinum drugs, available evidence supports the concept that DDP enters the cell, is distributed within the cell, and is exported by mechanisms that evolved to manage Cu homeostasis.

Zinc and the common cold

14.H.1
Zinc and the common cold: aspects of solution chemistry

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Common colds were shortened by 7 days in a 1984 clinical trial using zinc gluconate throat lozenges each 2 h. Between then and now, other double-blind, placebo-controlled clinical trials showed widely varying results. Reanalysis of these trials presents solution chemistry methods to elucidate differences in efficacy. Statistically significant correlation was shown between total daily dosages of positively charged zinc species and reductions in median (p=0.005) and mean duration (p<0.02) of common colds in these trials. Positively charged zinc species could shorten colds by 5 to 7 days, neutrally charged species had no effect on duration or severity, and negatively charged species could lengthen colds by up to 4.5 days. The biologically closed mouth–nose electric circuit influences the incidence of common cold occurrence.

14.H.2

Treatment of the common cold with zinc: effect on pro-inflammatory cytokine (soluble interleukin-1 receptor antagonist) and ICAM-1

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Adults and children in the United States experience two to six colds per year. Evidence that zinc is an effective therapy for colds is inconsistent. We conducted a study to test the efficacy of zinc acetate lozenges in reducing the duration of symptoms of the common cold. We conducted a randomized, double-blind, placebo-controlled trial. Fifty ambulatory volunteers were recruited within 24 h of developing symptoms of the common cold. Participants took one lozenge containing 13.3 mg of zinc as acetate or placebo every 3 h while awake as long as they had cold symptoms. Compared with the placebo group, the zinc group had shorter mean overall duration of cold symptoms (3.5 vs 7.4 days, $p<.001$), cough (2.1 vs 5.3 days, $p<.001$), and nasal discharge (3.0 vs 4.7 days, $p=.02$). Mean changes in soluble interleukin-1 receptor antagonist level and interstitial cell adhesion molecule (ICAM-1) decreased in zinc group and differed significantly from the placebo group. Mean changes in plasma zinc levels were higher in the zinc group and differed significantly from the placebo group. In conclusion, administration of zinc lozenges was associated with reduced duration of cold symptoms. Improvement in clinical symptoms with zinc treatment may be related to a decrease in pro-inflammatory cytokine levels. A decrease in ICAM-1 levels suggest that zinc may have decreased the binding sites for cold viruses to the endothelium.