ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI SCIENZE MEDICHE VETERINARIE

UNIMORE

A.I.S.E.T.O.V.

FESTEM

2018 SCIENTIFIC MEETING

ITALIAN ASSOCIATION FOR THE STUDY OF

TRACE ELEMENTS IN LIVING ORGANISMS - AISETOV

Ozzano Emilia, Bologna - October 12, 2018

Department of Veterinary Medical Sciences DIMEVET
***Alma Mater Studiorum*, University of Bologna**

The role of trace elements in health: from healthy environments to healthy living organisms

ABSTRACT BOOK

Organized by:

AISETOV, UNIBO and UNIMORE

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Italian Association for Study of Trace Elements in Living Organism (AISETOV)

Department of Veterinary Medical Sciences, University of Bologna

Department of Biomedical, Metabolic and Neural Sciences – Section of Public Health University of Modena and Reggio Emilia

With the patronage of

Federation of European Societies on Trace Elements and Minerals (FESTEM)

Italian Society of Hygiene, Preventive Medicine and Public Health

October 2018

SCIENTIFIC TOPICS

- Trace elements in wild and farm animals
- Trace elements in conventional and non-conventional food for humans and animals
- Trace elements in human health
- Methods for trace elements analysis

ORGANIZING COMMITTEE

Gloria Isani (President), University of Bologna

Giulia Andreani, University of Bologna

Emilio Carpenè, University of Bologna

Tommaso Filippini, University of Modena and Reggio Emilia

Paola Roncada, University of Bologna

VENUE

The meeting will be held on October 12th, 2018 from 9:00 to 17:00 in Aula Gherardini of the Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di sopra, 50 – 40064 Ozzano Emilia, Bologna.

The Department is located in Ozzano dell'Emilia, a small town 13 km East from Bologna along the old Roman road Via Emilia. In the first half of the 2nd century, BCE Romans founded the town of Claterna near Ozzano. This town grew to dimension comparable with Bononia (Bologna). Now close to the Department is possible to visit the recent archaeological excavations. Of special interest is a *domus* from the imperial period.

Meeting Program

08.30-09.30	Participants arrival and registration
09.00-09.30	**Welcome address and meeting opening**
	Welcome address, Giuliano Bettini, Director of DIMEVET
	Meeting opening, Paola Borella, Marco Vinceti
09.30-11.00	**Session I – Room Gherardini** Chairpersons: Emilio Carpenè and Laura Chiarantini
09.30-10.00	O-01 *Gastropod metallothioneins* Reinhard Dallinger
10.00-10.20	O-02 *The exhausting of metallothionein responses in the bivalve mollusks depending on the tense of exposure* Oksana Stoliar
10.20-10.40	O-03 *Involvement of different protein thiol groups in cadmium toxicity on hemoproteins* Giancarlo Falcioni
10.40-11.00	O-04 *Trace metals and metal-binding proteins in seaweeds and cyanobacteria used as nutritional supplement* Enea Ferlizza
11.00-11.30	***Coffee break***
11.30-12.50	**Session II – Room Gherardini** Chairpersons: Giancarlo Falcioni and Gloria Isani
11.30-11.50	*O-05 Bioremediation of contaminated soil: from in vitro to field model* Margherita Ferrante
11.50-12.10	O-06 *Evaluation of Pb and Hg levels in wild migratory water-birds nesting in different areas of Po Delta* Alessandro Guerrini
12.10-12.30	O-07 *Bacteria-produced ferric exopolysaccharide nanoparticles to improve the Tuber borchii mycorrhized plants performance* Laura Chiarantini
12.30-12.50	O-08 *Severe inflammations related to ketosis during transition period affect the plasmatic concentrations of Zn and Se in dairy cows* Fiorenzo Piccioli Cappelli
12.50-14.30	**Lunch and Poster Session**
14.00-14.30	**AISETOV member meeting – Room Gherardini**

14.30-16.30	**Session III – Room Gherardini** Chairpersons: Paola Borella and Marco Vinceti
14:30-14:50	O-09 *Metal/metalloid levels in cerebrospinal fluid in the bulbar- and spinal-onset amyotrophic lateral sclerosis* Maria Fiore
14.50-15.10	O-10 *Study of attentional disorders (unilateral spatial neglect) induced in school children exposed to environmental pollution in the Drâa Lasfer mining zone of the city of Marrakech* Sana Maidoumi
15.10-15.30	O-11 *Mercury in hair of children: effects on neurological performances* Anna Pino
15.30-15.50	O-12 *Lead exposure in children: an update on health effects* Paola Borella
15.50-16.10	O-13 *Analysis of intracellular Zn-hydroxyapatite nucleation in the early stages of human osteogenic differentiation combining synchrotron-based and diffraction techniques* Alessandra Procopio
16:10-16:30	O-14 *Trace elements in "unconventional food"* Emilio Carpenè
16.30-17.00	**Closing remarks**

CHAIRS AND SPEAKERS

Paola Borella, University of Modena and Reggio Emilia - FESTEM President

Emilio Carpenè, University of Bologna

Laura Chiarantini, University of Urbino Carlo Bo

Reinhard Dallinger, University of Innsbruck

Giancarlo Falcioni, University of Camerino

Enea Ferlizza, IZSLER, Bologna

Margherita Ferrante, University of Catania

Maria Fiore, University of Catania

Alessandro Guerrini, University of Bologna

Gloria Isani, University of Bologna

Sana Maidoumi, Cadi Ayyad University

Fiorenzo Piccioli Cappelli, Università Cattolica Sacro Cuore, Piacenza

Anna Pino, Italian National Institute of Health, Rome

Alessandra Procopio, University of Bologna

Oksana Stoliar, University of Ukraine

Marco Vinceti, University of Modena and Reggio Emilia - AISETOV President

Oral Presentations

O-01. Gastropod Metallothioneins

Reinhard Dallinger[1], Oliver Zerbe[2], Mercé Capdevila[3], Òscar Palacios[3], Ricard Albalat[4]

[1]*Department of Zoology, University of Innsbruck, Austria*
[2]*Department of Chemistry, University of Zürich, Switzerland*
[3]*Department of Chemistry, Autonomous University of Barcelona, Spain*
[4]*Department of Genetics, Microbiology and Statistics, University of Barcelona, Spain*

The first Metallothionein (MT) was isolated form the horse kidney as a Cys-rich protein naturally associated Cd^{2+} and Zn^{2+}. Since then, MTs were discovered in a vast number of animals, and in many plants, fungi and bacteria, too. The high stability constants between MTs and transition metal ions (Cd^{2+}, Zn^{2+}, Cu^{+}) at neutral pH with values between 10^{-19} – 10^{-12} M^{-1} suggest that metal binding is a primary task of MTs. In spite of this, the physiological functions ascribed to these proteins *in vivo* seem often to be decoupled from their metal binding properties. Evidently, this makes it impossible and inappropriate to link the activity of MTs as a protein family to one particular metal related to a specific function.

This apparent dilemma could possibly be solved if we try to understand the functional relationships of MTs in an evolutionary context, by extending our focus on MTs of "unconventional" animal lineages with MT model systems in which the relationship between structural features, metal binding behavior and biological functions is more evident and can be traced across genetically related taxa. The animal clade of Gastropoda may most likely possess such a system.

In our recent studies we can show that in a number of major gastropod lineages (slugs, winkles and snails), increased Cd tolerance is linked to convergent evolution of Cd-specific MTs. This specificity for Cd has a structural basis, discriminating at the same time the association of these MTs with Zn^{2+} and Cu^{+} ions *in vivo*. This predetermines these MTs for Cd detoxification. Upon repeated transition of gastropod lineages to life on land and freshwater, however, Cd-specificity has been subjected to diversification and modifications, with the emergence of Cu-specific MT isoforms serving Cu homeostasis in some land snails, up to the complete loss of metal-specific binding features in some freshwater snails.

O-02. The exhausting of metallothionein responses in the bivalve mollusks depending on the tense of exposure

Oksana Stoliar[1], Olena Mishchuk[2]

[1]*Department of Chemistry and the methods of its teaching, Volodymyr Hnatyuk Ternopil National Pedagogical University, Ukraine*
[2]*Rowan University, Glassboro, NJ, USA*

The evaluation of metal accumulation in metallothioneins (MTs) of bivalve mollusks is usually used for the bioindication of environmental pollution by toxic metals. However, containing a remarkable number of thiol groups, MTs could also act as scavengers of reactive oxygen species. The aim of this study was to validate the functionality of MTs of bivalve mollusks by comparing the responses in experimental and field exposures. Both native and transplanted for 30 days bivalves Anodonta cygnea were used at three different sites of a small tributary in the basin of the Dnister river along a gradient of pollution: in a village (Kr), near a motorway (MW), and in the municipal effluents area (Tr). The mollusks for laboratory exposures and transplantations were collected in the pristine site. In the laboratory, the mussels were exposed to Cu2+ (0.01 and 0.2 mg L-1) for 14 days. In the mollusks transplanted at Kr site and exposed to 0.01 mg·L-1 of Cu, a hormesis-like response was detected: an increase in the concentrations of MTs, Cu- and Zn-MT (threefold) and the GSH levels, and a decrease in the lipid peroxidation (LPO). The Zn/Cu ratio in MTs was approximately 2:1 in these groups. The specimens from the polluted sites (MW, Tr), both native and transplanted, and whose exposed to 0.2 mg L-1 Cu demonstrated the exhausting of particular MT responses (concentration and profile under the ion-exchange chromatography), and increases in the levels of GSSG/GSH ratio and LPO. The Zn/Cu ratio in their MTs was 1:1. Hence, the up-regulation of MTs functionality was correspondent to the changing of their metal composition and accompanied with the activation of antioxidant response. The activation or exhausting of MTs responses could potentially be utilized in the ranking of environmental impacts.

O-03. Involvement of different protein thiol groups in cadmium toxicity on hemoproteins

Patrick Orlando[1], Sonia Silvestri[1], Ilenia Cirilli[2], Fabio Marcheggiani[1], Luca Tiano[1], Giancarlo Falcioni[3]

[1]*Department of Life and Environmental Sciences, Università Politecnica delle Marche*
[2]*Department of Odontostomatologic and Specialized Clinical Sciences, Università Politecnica delle Marche*
[3]*Scuola di Scienze del Farmaco e dei Prodotti della Salute, University of Camerino*

Introduction: Cadmium (Cd) is generally considered a non-essential trace element ubiquitously present in the terrestrial and aquatic environments. It has been investigated mainly for its toxicity in several species belonging to different taxa. The toxicity of Cd is mostly linked to cancerogenesis through mechanisms acting at different molecular levels (Chen et al., 2016), including the inhibition of DNA repair (Whiteside et al., 2010) and modulation of apoptosis (Liu et al., 2009). Moreover Cd, can bind to sulfhydryl groups of proteins causing their depletion also involving protein denaturation. The aim of this study was to detect the ability of cadmium to induce precipitation in hemoptroteins that present a different number and position of cysteines. These determinations could be useful to better understand the mechanism involved in the protein stability due to excessive metal exposure. Within this perspective, we have started an investigation using myoglobin, human haemoglobin and the purified haemoglobin components from trout (*Oncorhynchus mykiss*).
Methods: Horse heart myoglobin was reduced by the addition of dithionite and the excess was removed by the passage of the solution through a Sephadex G-25 column equilibrated with 50 mM phosphate buffer, pH 7.0. Human haemoglobin (HbA) and trout haemoglobin components (HbI and HbIV) was obtained as previously described (Rossi Fanelli et al. 1961; Binotti et al. 1971). The effect of cadmium on the haemoprotein stability was monitored spectrophotometrically for 8 hours at 700 nm during incubation of solutions containing 1 mg/mL Hb in presence of different amounts of cadmium chloride and in phosphate buffer pH 7.8 at 37°C.
Results and conclusions: Results highlight an accelerating effect of cadmium on the time course of precipitation evident only for HbIV that could have been prevented by glutathion coincubation in a dose dependent manner. Experiments carried out in presence of para mercurium benzoate (PMB), a molecule able to specificly react with free thiolic groups, showed very similar behaviour. From sequence analysis, it is evincible that myoglobins, insensitive to cadmium destibilizing effect, generally do not contain cysteines; while these are always present in hemoglobins. In general, the hemoglobins do not form intra molecular disulphide bridges and the cysteines are therefore present as sulphydryls. Notably HbI was not affected by cadmium exposure differently from HbIV; in this respect, different location and shorter distance of cysteines in the latter could play a role in the formation of molecular bridges by linking the bivalent cadmium with the two sulphydryl groups positionated in G11 and G15 promoting HbIV proness to precipitation.

O-04. Trace metals and metal-binding proteins in seaweeds and cyanobacteria used as nutritional supplement

Enea Ferlizza[1], Simonetta Menotta[1], Silva Rubini [2], Domenico Gigliotti[1], Giulia Andreani[3], Martina Bertocchi[3], Giorgio Fedrizzi[1], Gloria Isani[3]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento Chimico, Bologna*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione di Ferrara*
[3]*Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna*

Introduction: For centuries, local populations around the world used seaweeds and cyanobacteria as food. In fact, these aquatic organisms contain high concentrations of proteins, vitamins and minerals, in particular essential trace elements. The consumption of seaweeds in Western countries showed an increase in recent years, raising concern about their quality and safety. The purpose of this preliminary research was to analyze trace metal concentrations and to isolate metal-binding proteins in commercial samples of seaweeds and cynobacteria used as human and animal nutritional supplementation.
Material and Methods: Sixty samples of dry and fresh edible seaweeds and cyanobacteria were obtained from the market. Nineteen essential and non-essential trace elements (Mn, Fe, Co, Cu, Zn, Se, Mo, Cr, V, Pb, Cd, Hg, As, Al, Ag, Ni, TI, U, Sb) were analyzed by ICP-MS after mineralization. Cytosolic proteins were separated by gelfiltration chromatography.
Results and Discussion: Trace element concentrations are in the range of those reported by other authors (Desideri et al., 2016) and vary depending on the species, the environmental conditions and the water content of the commercial products. In particular, Fe concentrations in fresh samples ranged from 6 to 95 μg/g. In cyanobacteria, a mean Fe concentration of 643 μg/g dw was measured, resulting higher than those generally found in food of animal and plant origin. Very low concentrations of Cd, Pb and Hg were determined in all the examined samples, while Al showed wide variations and raised concern due to high values found in dry samples of *Ulva sp*. Despite As concentrations were high in some dry samples, this toxic element should rise less concern, because it was present mainly as organic As. The amount of Fe and Zn bound to cytosolic proteins was one order of magnitude higher in cyanobacteria than in macroalgae, indicating a possible higher bioavailability of these trace elements in the former organisms.

References

Desideri D, Cantaluppi C, Ceccotto F, M. A. Meli, C. Roselli, and L. Feduzi, Essential and toxic elements in seaweeds for human consumption, J. Toxicol. Environ. Chem. (A), 2016, 79, 112–122.

Acknowledgements

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O-05. Bioremediation of contaminated soil: from in vitro to field model

Margherita Ferrante, Gea Oliveri Conti, Cristina Restuccia, Sebastiano Cosentino, Giovanni Mauromicale, Chiara Copat, Alfina Grasso, Maria Fiore, Pietro Zuccarello, Antonio Cristaldi

Department "GF Ingrassia", University of Catania, Catania, Italy

Intentional or accidental introduction into the soil of pollutants can lead to serious risk for the environment and public health. Different techniques are been used for soils remediation, within phytoremediation has been proposed as an environmentally friendly and cheaper alternative.
We have tested *in vitro* the bioaccumulation capacity of *Trichoderma harzianum*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus* for Ni, Cd, Cu, As, Zn, Pb, V, Hg, and Policiclic haromatic Hydrocarbons (16 EPA priority). *T. harzianum* was evaluated as a suitable microorganism for the continuation of the our experimentation. *Arundo donax* was the plant species used for tests of controlled growth in greenhouses. The samples of *A. donax* and *A. donax* mycorrhized with *T. harzianum* were exposed to the heavy metal mix, and to PAH mix and the analyzes were carried out according to EPA method 6020b:2014 with ICP-MS Elan DRC-e Perkin Elmer for metals and according to APAT IRSA method with HPLC UV/FL Varian ProStar 325 for PAH.
.A. donax and *A. donax* mycorrhized with *T. harzianum* were exposed to two different exposure doses of a heavy metal mix and of an IPA mix, for a total time of 7 months in sperimental greenhouses.
A. donax and *A. donax* mycorrhized with *T. harzianum* did not suffer at the physiological level of the effect of the contaminants, rather especially mycorrhised plant showed a good ability to phytostabilize and to extract the heavy metals and to phytodegradate the PAHs, in relation to the duration of the implant too. This suggests to deepen and strengthen the intervention mechanisms by the organisms employed, increase the number of exposure tests to major contaminants, perform field tests, evaluate the possible reuse of the produced biomass.

O-06. Evaluation of Pb and Hg levels in wild migratory water-birds nesting in different areas of Po Delta

Alessandro Guerrini[1], Giorgio Fedrizzi[2], Alessandro Andreotti[3], Luisa Iannone[1], Paola Roncada[1]

[1]*Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento Chimico, Bologna*
[3] *ISPRA - Istituto Superiore per la Protezione e la Ricerca Ambientale, Ozzano Emilia, Italy*

The use of fossil fuels as main energy resource led to metal emissions that spread all over the environment and contaminate ecosystems and food. This has resulted in even serious toxic effects, both in humans and animals. A characteristic feature of some metals is their pronounced biological accumulation and biomagnification in food chains. For these reasons, their monitoring in the environment should be constant over time. This study shows data on Pb and Hg levels in tissues of various species of wild migratory water-birds nesting in different areas of Po Delta. The sampling was carried out from May to July 2016. Analyses were performed according to the internal test method of the IZSLER by inductively coupled plasma mass spectrometry (ICP/MS). The two metals were found in most of the analyzed samples. The maximum levels of Pb were found in the natal down of nestlings (3.5515 mg/kg), and in feathers, femur and humerus of adult birds (5.2363, 15.469 and 12.9760 mg/kg, respectively). In adults the highest concentrations of Hg were found in feathers and liver (6.0144 and 6.1939 mg/kg, respectively) while in nestlings the highest level was found in down (4.9411 mg/kg). The differences between the average concentrations detected in adults and those measured in nestlings can be explained by chronic exposure to metals. This exposure is usually highest at the top of food chains in predatory species. Thus, the birds of prey may be suited for monitoring purposes, but their sampling may be difficult due to their low population densities and high mobility. Moreover, in the present study only birds dead of natural causes were used. In the future, the analysis of a higher number of samples will provide a more complete picture.

Acknowledgments
Authors greatly acknowledge Fabrizio Borghesi, Lorenzo Serra, Stefano Volponi and Alberto Altafini for their collaboration.

O-07. Bacteria-produced ferric exopolysaccharide nanoparticles to improve the *Tuber borchii* mycorrhized plants performance

Pamela Leonardi[1], Franco Baldi[2], Laura Chiarantini[3], Livia Vittori Antisari[1], Mirco Iotti[4], Filippo Piana[1], Federico Lugli[5], Alessandra Zambonelli[1]

[1]*Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy*
[2]*Department of Molecular Sciences and Nanosystems, Cà Foscari University, Mestre Venice, Italy*
[3]*Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy*
[4]*Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy*
[5]*Department of Cultural Heritage, University of Bologna, Ravenna, Italy.*

Truffles are hypogeous fungi belonging to the genus *Tuber* (Pezizales, Ascomycetes), which live in ectomycorrhizal association with the roots of specific host plants. The cultivation of truffles involves the production of seedlings mycorrhized with *Tuber* spp. in greenhouse which should be then transplanted in specific calcareous soils. One of the prerequisites for the success of truffle production is the quality of the plants produced in greenhouse, which should be healthy and extensively mycorrhized with *Tuber* spp. In this work we tested the effect of Fe(III) exopolysaccharide nanoparticles (Fe-EPS), biogenerated by *Klebsiella oxytoca* DSM 29614 under anaerobic conditions, on *Quercus robur* seedlings inoculated with *T. borchii* in greenhouse. Fe-EPS were able to limit the chlorosis caused by high soil content of $CaCO_3$ during the first few months of seedling growth and increased *T. borchii* mycorrhizal colonization. These results are very promising for using Fe-EPS in truffle cultivation both in greenhouse and in iron-depleted soils.

O-08. Severe inflammations related to ketosis during transition period affect the plasmatic concentrations of Zn and Se in dairy cows

Fiorenzo Piccioli-Cappelli, Matteo Mezzetti, Andrea Minuti, Erminio Trevisi

Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti (DIANA), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy

The severe negative energy balance and the occurrence of inflammatory phenomena makes the transition period (TP) as the most critical phase for dairy cows. In fact, when unrestrained, those phenomena could impair health conditions and reduce performance of dairy cows. Variation in plasma levels of trace elements could have a role in the regulation of these processes.13 Holstein cows were housed in individual tied stalls and blood samples were collected from -28 to 14 days from calving (DFC) to assess a wide hematochemical profile. Cows have been retrospectively divided in 2 groups according to their β-hydroxybutirate levels, assuming 1.4 mMol/L as a threshold of subclinical ketosis: Ket (6 cows) and Ctr (7 cows). Ket showed higher level of Interleukin-1β before calving respect to Ctr cows (246 vs 101 pg/mL $P<0.01$), indicating different immune competence. The blood Zn level showed a more severe reduction at calving in Ket and then remained at lower level after calving (8.80 vs 11.5 mcMol/L, $P<0.05$). This suggests a blockage by the liver due to a more severe inflammation in Ket cows, according also with the higher level of ceruloplasmin (2.75 vs 2.25 mcMol/L, $P<0.05$). Plasmatic Se resulted similar between groups at -28 DFC. Thereafter Ket showed a marked reduction at calving (1.0 vs 1.2 mcMol/L, $P=0.12$) and lower concentrations after calving respect to Ctr (1.28 vs 1.45 mcMol/L, $P=0.08$). Se and Zn are cofactors in enzymes implicated in the abatement of reactive oxygen metabolites (ROM). Thus, their low levels in plasma may increase the exposure to oxidative stress. This hypothesis can be supported by the higher concentration of ROM in Ket cows (17.8 vs 13.9 mgH_2O_2/dL, $P<0.05$) observed after calving. The plasma reduction of Se and Zn could partly due to the lower feed intake in Ket and could be reduced feeding highly bioavailable supplements.

O-09. Metal/metalloid levels in cerebrospinal fluid in the bulbar- and spinal-onset amyotrophic lateral sclerosis

Maria Fiore, Francesco Patti, Paladino Grazia, Emanuele D'Amico, Salvatore Lo Fermo, Alfina Grasso, Chiara Copat, Mario Zappia, Margherita Ferrante

Department "GF Ingrassia", University of Catania, Catania, Italy

Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of the central nervous system (CNS) that causes progressive and irreversible damage in motor neurons. Different causal hypotheses include genetic, viral, traumatic and environmental mechanisms, such as exposure to heavy metals, that interact with each other.
The aim of this study was to investigate metal/metalloid levels in cerebrospinal fluid (CSF) in a well-defined cohort of ALS patients, to analyze differences due to gender or clinical onset, focusing on bulbar o spinal ones.
Material and Methods: Inductively coupled plasma mass spectrometry (ICP-MS) was used to quantify Cu, Se, Fe, Zn, As, Co, Hg, Cd levels. Statistical analysis was conducted using IBM SPSS Statistics 21.0.
Results: Thirty-seven patients were enrolled (62.2% women), median age of 70 years (IQR: 65-78 years), no significant difference was found between the age of men and women. Thirty-one (83.8%) patients had a spinal onset and 6 (16.2%) a bulbar onset, there was no significant association between type of onset and gender (p-value: 0.593). None of the metals showed different levels between males and females, whereas Cu and Mn levels were higher in patients with spinal onset than in patients with bulbar onset (Cu: 129.8 vs 29.8; p-value: 0.007. Mn: 3.4 vs 1.8; p-value: 0.026). A positive correlation between age and V was found ($r=0.381$, $p = 0.020$) and a negative correlation between age and As ($r = - 0.330$, $p = 0.046$).
Conclusion: These results support the hypothesis that metals with neurotoxic effects are involved in the pathogenesis of ALS, with relevant differences between bulbar and spinal onset. In accordance with previous studies these findings showed statistically significant associations between spinal onset of ALS and exposures to metals, in particular Cu and Mn. It is suggested that future epidemiological research considers separately the two forms of ALS.

O-10. Study of attentional disorders (unilateral spatial neglect) induced in school children exposed to environmental pollution in the Drâa Lasfer mining zone of the city of Marrakech

Sana Maidoumi[1], Hajar Sebban[1], Hind Ait Belcaid[1], Mohamed Loukid[2], Nadra Lekouch[1], Ahmad Omar Tohami Ahami[3], Azeddine Sedki[1]

[1]*Laboratory of Hydrobiology, Ecotoxicology, Global Sanitation and Change. Department of Biology, Faculty of Sciences - Semlalia. Cadi Ayyad University, Marrakech, Morocco*
[2]*Laboratory of human ecology. Department of Biology, Faculty of Sciences - Semlalia. Cadi Ayyad University, Marrakech, Morocco*
[3]*Laboratory of Biology and Health. Unit of Neuroscience and Nutrition. Department of Biology, Faculty of Science. Ibn Tofail University, Kenitra, Morocco*

Like deficits affecting overall measures in children, lead exposure leads to more specific deficits suggestive of attention deficit disorder.
Objective: To evaluate the visual attention function among schoolchildren living in a mining area compared to a control area in Marrakech.
Methods: We used the Bell Test (Gauthier et al., 1989) or Bell Dam Test (CBT), for the assessment of visual attention and unilateral spatial neglect (NSU) among children in school at the first and second level of basic education. Interviews with parents were conducted to identify co-variables significantly related to lead exposure.
Results: We deduced that attention is affected in these children. The omission of six bells or more to the right or left of the TBC page was found in 88.5% of children with a profile of unilateral negligence on the right side (2.5%). The scanning method chosen was disorganized in 32.5% of them. Our results are similar to those recently presented by EL Azmy et al. (2014) in children in the Mrirt area and agree with Gauthier et al. (1989) who showed that the strategy of scanning hemiplegic left is disorganized in comparison with that of normal.
Conclusion: It should be noted that a visual attention deficit could be related to a dysfunction in the posterior associative cortex in the temporal and parietal lobes of these children following exposure to lead.
Key words: visual attention, visuospatial negligence, environment, lead, child, brain dysfunction.

O-11. Mercury in hair of children: effects on neurological performances

Anna Pino, Oreste Senofonte, Francesco Petrucci, Giovanni Forte,
Costanza Majorani, Beatrice Bocca, Alessandro Alimonti

National Health Institute, Rome, Italy

Exposure to different forms of Hg can have serious health consequences, among these methylmercury (MeHg) can have the most important toxicity risk to humans after exposure through consumption of fish. Once in the bloodstream, MeHg can accumulate in the brain causing neurotoxic effects by crossing the blood-brain barrier

Generally, more than the 80% of Hg in hair is as MeHg, which is taken up by hair follicles as MeHg-cysteine complexes. Thus, hair can be used for epidemiological studies to determine the total Hg concentrations as biomarkers for MeHg exposure.

In this context, hair samples were collected from 200 children (7 years) living in a site in the North East (A) and from 299 children (6-11 years) living in a urban area of South Italy (B) to determine the levels of MeHg.

To test the neurotoxicity of MeHg, children were subjected to cognitive and neuropsychological tests.

The hair values of MeHg in the children understudy were comparable with data reported in other international surveys. On the other hand, combining results of the neurological tests with MeHg levels, a possible relationship between MeHg and an increase of the errors average reported in some neurological tests has been noted, so

a possible neurological influence in children might not be excluded even though the MeHg levels were not elevated. The influence on neurological performances of the children of other determinants due to the family environment (socio economic status, educational level, etc.) were also investigated.

O-12. Lead exposure in children: an update on health effects

Paola Borella, Annalisa Bargellini, Isabella Marchesi, Angela Ferrari, Gianfranco Vivoli

Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena

The effects associated with lead exposure are still considered relevant, particularly for children, despite the implementation of numerous public policies and laws aimed to reduce the use of this toxic trace element. In the eighty years, in the Sassuolo district ceramics using Pb in the dyes, air contamination reached 10.3 µg/m3 and assumption with diet was higher than 600 µg/die. A large survey on Pb-exposed women highlighted higher rates of spontaneous miscarriage, other than dysmenorrhea, compared to controls. Among over 1,000 elementary school pupils, with an average of 17.5 µg / dl, the most exposed children had a significant increase in restlessness, distractibility and decreased scholar performance. Within an European Multicenter Study, the CNS effect of Pb in 300 children between 8 and 11 years old was evaluated, and Pb concentration in deciduous teeth was significantly associated with impairment of some cognitive functions. Other studies carried out on children aged 7-8 years old showed that the teeth Pb levels (mean 6.2 µg/g tooth) were negatively related with height. This relationship was also confirmed in teenagers. We suggested that no threshold level can guarantee the absence of detrimental effect on the child nervous system, a statement never denied, as nowadays numerous researchers suggest to adopt a zero tolerance in blood lead level. At present, Pb exposure is decreased and the neurological effects of lead in children are well established, but recent studies open other perspectives in this field, and the pernicious effects of lead at any measurable concentration have been emphasizes. A chronic low exposure with a blood concentration < 10 µg / dl is still associated with the development of neurobehavioral or renal effects, suggesting that also minimal concentration needs attention on the various extra and intracellular events accompanying the presence of this toxic in the human organism.

O-13. Analysis of intracellular Zn-hydroxyapatite nucleation in the early stages of human osteogenic differentiation combining synchrotron-based and diffraction techniques

Alessandra Procopio[1*], Alexandra Pacureano[2], Azzurra Sargenti[1], Cinzia Giannini[3], Davide Altamura[3], Concettina Cappadone[1], Giovanna Farruggia[1], Jeanette Maier[4], Stefano Iotti[1], Peter Cloetens[2], Emil Malucelli[1]

[1]*Dep of Pharmacy and Biotechnology, University Bologna, Italy, alessandra.procopio3@unibo.it*
[2]*European Synchrotron Radiation Facility, Grenoble, France*
[3]*Institute of Crystallography (IC), National Research Council, Bari, Italy*
[4]*Dipartimento di Scienze Biomediche e Cliniche "L. Sacco", University of Milan, Italy*

Introduction: All the body of knowledge about biomineralization (BM) is provided by studies on the advanced phases of biomineralization, which mainly occur in the extracellular matrix, while studies on the early stages of this process are scarce. Specifically, the accumulation of early hydroxyapatite (HA) crystals and the transport of early mineral to the extracellular matrix space are incompletely understood. Here, we investigate the early stages of biomineralization by evaluating the genesis of the initial mineral nuclei (MN) and their evolution toward hydroxyapatite, in human bone mesenchymal stem cells, exposed to an osteogenic cocktail for 4 and 10 days.
Material and Methods: Synchrotron X-ray fluorescence microscopy (XRFM) was carried out at the beamline ID16A (@ESRF, Grenoble FR) to determine the chemical composition of MN formation at nanoscale. Moreover, we acquired Phase- contrast tomography (15 nm) and X-ray Fluorescence Tomography to derive the elemental distribution and density over the sample thickness [1]. In order to reduce the radiation damage, we acquired Frozen Hydrated bMSC. We employed wide angle x-ray scattering (WAXS @ CNR – Istituto di Cristallografia, Bari IT) to gain information at atomic scale on the crystalline structure of the MN.
Results and Discussion: After 4 days of the osteoblastic induction, some nano MN formations are evident, where Ca, P and Zn are co-localized. These data indicate that MN formation starts early during the osteogenic differentiation of bMSC. The occurrence of a germinal Zn presence represents a novel finding providing experimental evidence about the Zn role in HA nucleation [2]. After 10 days of the osteogenic differentiation, the depositions are massive in term of both dimension and number. The 3D reconstructions of phase contrast images of bMSC cells at 10 days allow to browse the entire cell along its thickness to explore the three-dimensional localization of the MN. Several spots are localized in the cytoplasm of the cell, indicating that the BM starts in the intracellular environment. The same cells were scanned by X-ray fluorescence tomography to allow the 3D visualization of Ca, P and Zn elemental distribution. WAXS measurements revealed the hexagonal HA as the unique crystalline structure present in differentiating bMSC.
Conclusions: Thanks to the combination of 3D high resolution x-ray techniques, we demonstrate that BM starts with hydroxyapatite nucleation in the intracellular environment, rapidly evolving toward a hexagonal hydroxyapatite crystal very similar to the one present in human bone as detected after just ten days of osteogenic induction.

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O-14. Trace elements in "unconventional food"

Emilio Carpenè[1], Enea Ferlizza[2], Simonetta Menotta[2], Fabiana Cappi[2], Giulia Andreani[1], Giorgio Fedrizzi[2], Gloria Isani[1]

[1]*Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento Chimico, Bologna*

Introduction: The human diet is based on myriads of different sources of nutrients, including unconventional food from cyanobacteria to mammals. In most societies, the choice of food is related to historical, religious and psychological groundings that often limit the acceptance of unconventional and exotic meats (Cawthom and Hoffman, 2016). The aim of the present research was addressed to investigate the concentrations of non-essential and essential trace elements in"unconventional food" prepared from samples of venison, snails and mushrooms from the Quaderna Valley (Bologna).
Material and Methods: Thirty samples of different cooked food obtained from mammals, birds, snails and mushrooms were included in this study. Nineteen essential and non-essential trace elements (Mn, Fe, Co, Cu, Zn, Se, Mo, Cr, V, Pb, Cd, Hg, As, Al, Ag, Ni, TI, U, Sb) were analysed by ICP-MS after tissue mineralization.
Results and Discussion: Trace element concentrations varied widely depending on the investigated samples. Among non-essential elements, the highest levels of Pb were found in samples of woodcock, *Scolopax rusticola* (2.4 µg/g wet wt) and teal, *Anas crecca* (4.8 µg/g wet wt). These high concentrations are related to Pb microscopic fragments present in the meat of venison obtained by local hunters. Meanwhile, in other venison samples from pheasant (*Phasianus colchicus*) and hare (*Lepus europaeus*) Pb concentrations were significantly lower ($p<0.05$), probably due to the different recipe used for meat cooking. The highest Cd concentration (0.4 µg/g wet wt) was found in one sample of Roman snail, *Helix pomatia*, this finding is confirmed by additional analysis on fresh snails. Mushrooms showed Hg levels as high as 0.5 µg/g wet wt. As regards essential elements, in general all the investigated samples were rich in Fe, Zn and Cu. In particular, snails had concentrations of Cu one-two order of magnitude higher, due to the presence of hemocyanin.

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Posters

P-01. Interaction between copper and temperature in the gills of *Mytilus galloprovincialis*: expression of metallothioneins and antioxidant enzymes

Paola Irato, Arianna Maschio, Veronica Rizza, Ilaria Schettino,
Gianfranco Santovito, Laura Tallandini

Department of Biology, University of Padua

It is well known that Cu is a very important essential trace metal in all organisms, but at elevated levels it is one of the most toxic heavy metals. It is largely accepted that, when the homeostasis of the metal is compromised, the Cu toxicity is caused from the capacity of Cu ions to produce oxidative damage, inducing the formation of reactive oxygen species (ROS).
Keeping in mind that Cu is one of the most widespread contaminants in the marine environment and considering the risks of climate change, it seemed interesting to investigate the physiological responses of marine organisms to combined exposures to high Cu concentration and different temperatures. In this study, specimens of the bioindicator organism *Mytilus galloprovincialis*, were exposed to 0.050 ppm Cu in seawater at 15, 20 and 25°C for 96h. In parallel other specimens were exposed, in clean water, at the same temperature conditions for 96h.
All the treated organisms were previously acclimatized for a month at a temperature of 20°C, which can be considered as the physiological temperature. The studied biomarkers were metallothionein (MT) concentration and catalase (CAT) and glutathione peroxidase (GPX) activities, analyzed in gills.
MT expression is induced by the Cu-treatment only at 15°C.
The enzymatic activity of CAT is lower at 15 and 25°C than at 20°C, both in Cu untreated and in treated specimens: the activity of this enzyme appears to be affected by the changes in temperature and not by the Cu treatment.
The GPX activity in Cu untreated specimens correlates with the temperature, while in the Cu treated seems to decrease: the activity of this enzyme appears therefore to be affected by both the temperature and the Cu exposure.
In conclusion, only for the GPX activity seems to be affected by a combination of Cu and temperature change.

P-02. Accumulation of cadmium, copper and zinc in the bivalve mollusks from the reservoirs of power plants

Oksana Stoliar[1], Gunta Spriņģe[2], Lesya Gnatyshyna[1,3], Vira Homa[1], Oksana Horyn[1], Agnija Skuja[2], Ivars Druvietis[2], Dāvis Ozoliņš[2], Ilga Kokorite[2], Viktoria Martinyuk[1], Halina Falfushynska[1], Lyubomir Tsaryk[1], Natalya Mischuk[1]

[1]*Volodymyr Hnatyuk Ternopil National Pedagogical University, Ukraine*
[2]*University of Latvia, Latvia*
[3]*I.Ya. Horbachevsky Ternopil State Medical University, Ukraine*

Power plants (PPs) (nuclear, fuel or hydropower, NPPs, TPPs and HPPs correspondingly) are equipped with reservoirs that accumulate toxic effluents and have elevated temperature of water (NPPs and TPPs) or disturb the water flow (HPPs). The aim of this study was to evaluate the impact of the PPs on the accumulation of metals (Zn, Cu, Cd) in the bivalve mollusks. Specimens of *Unio tumidus* in Ukraine and *Dreissena polymorpha* in Latvia were sampled in the reservoirs of two TPPs and one NPPs, before and after dams of two HPPs at the tributaries of the river Dniester and Pripyat (upper streams) (Ukraine) and in the reservoir of Riga HPP on the river Daugava (Latvia) and pristine ponds in both countries. The concentrations of metals in the metallothioneins (MTs) and thiols (MT-SH, GSH&GSSG) were determined in the soft tissues. Expectedly, the mollusks from TPPs accumulated highest concentrations of Zn and Cu but not Cd. In the MTs, highest concentration of Cu was detected in the mollusks from NPP in accordance with its high level in the water. However, the level of Zn and Cd in the MTs was highest in the pristine sites. The level of MT-SH in Unionidae mollusk was highest among all studied groups in the mollusks from NPP and lowest in the mollusks from both TPPs. For the *D. polymorpha*, the difference in the MT-SH levels between the sites was not found, but the level of GSH and GSH/GSSG ratio was lower in the mollusks from the reservoir. The ratio of MT-SH to metalated (Zn, Cu, Cd)-MTs was highest in the mollusks from the TPPs despite these reservoirs were highly polluted by metals. To summarizing, the inability of MTs to bind the toxic metals reflected the common impact of chemical and heat pollution in the reservoirs of PPs.

P-03. Titanium chloride and titanium oxide nanoparticles cause metallothionein hypermetalation in a single and combine with bisphenol A exposures

Lesya Gnatyshyna[1,2], Halina Falfushynska[1], Oksana Horyn[1], Vira Homa[1], Olena Mishchuk[3], Oksana Stoliar[1]

[1]*Volodymyr Hnatyuk Ternopil National Pedagogical University, Ukraine*
[2]*I.Ya. Horbachevsky Ternopil State Medical University, Ukraine*
[3]*Rowan University, Glassboro, NJ, USA*

The major direction of the utilizing of nano-TiO_2 (n-TiO_2) is relating to its unique properties of photocatalist of organic substances. This activity is applied in wastewater purification due to relatively low cost and high stability. Therefore, the aim of this study was to evaluate the biological responses to n-TiO_2 in the co-exposure with phenol compound bisphenol A (BPA) that is most widely used chemicals in commerce. Since the known signs of the n-TiO_2 and BPA toxicity are associated with the oxidative damage (n-TiO_2) and metabolic disorders (BPA), the focus in this study was put on the metallothionein (MT) metal binding and potential scavenging activities of cellular thiols. The specimens of bivalve mollusk *Unio tumidus* were subjected to 14-day exposure to n-TiO_2 (1.25 μM), BPA (0.88nM), n-TiO_2+BPA, or $TiCl_4$ (Ti, 1.25 μM, as a positive control for n-TiO_2). The concentration of MT was detected from the level of thiols and metal concentrations (Zn, Cu). Exposure to all Ti-contained compounds (single and combine) resulted in the elevated metalation of MT without the increase in their thiol concentration (MT-SH) and increase in the lactate/pyruvate ratio. However, the oxidative stress responses were distinct. While Ti induced oxidative stress (increased superoxide dismutase activity and ROS generation), n-TiO_2 in the single exposure caused the down-regulation of ROS and increasing of GSH level by two. In opposite, co-exposure to n-TiO_2 and BPA caused the depletion of MT-SH and GSH and decreasing in the GSH/GSSG (by three) and lactate/pyruvate ratios. Hence, thiols were not the specific molecular targets for Ti and n-TiO_2. The unusual state of MT supermetalation in all Ti-related exposures could be related to the particular properties of Ti (IV), which coordinates with proteins and nucleic acids through oxo-bridges. In any case, this property of Ti(IV), could confirm its specific behavior exploring in the anticancer treatment.

P-04. Biomarker responses to exposure of trace metals and a thiocarbamate fungicide in the pond snail *Lymnaea stagnalis*

Lesya Gnatyshyna[1,2], Halina Falfushynska[1], Oksana Stoliar[1], Reinhard Dallinger[3]

[1]*Research Laboratory of Comparative Biochemistry and Molecular Biology, Ternopil National Pedagogical University, Ukraine*
[2]*Department of General Chemistry of I.Ya. Horbachevsky Ternopil State Medical University, Ternopil, Ukraine*
[3] *Department of Zoology, University of Innsbruck, Innsbruck, Austria*

Freshwater pulmonate mollusks are commonly used for the study of neurotoxic effects and developmental disorders. Their biochemical responses to toxic substances have so far been studied to a much lesser extent. The goal of the present study was to evaluate the involvement in metal homeostasis and stress response of metallothioneins and a number of cellular biomarkers in the *Lymnaea stagnalis* upon exposure to typical environmental pollutants, including metals and a zinc and manganese containing thiocarbamate fungicide, called TATTOO (Bayer CropScience Inc., Alberta, Canada). The snails were subjected to copper (Cu^{2+}, 10 $\mu g \cdot L^{-1}$), zinc (Zn^{2+}, 130 $\mu g \cdot L^{-1}$), cadmium (Cd^{2+}, 15 $\mu g \cdot L^{-1}$) and the fungicide TATTOO (mixture of propamocarb and mancozeb (91 $\mu g\ L^{-1}$) during 14 days.
All exposures to metals caused the elevation of the concentration of metals (Zn, Cu or Cd) in the tissues and the level of metallothioneins detected from their thiols (MT-SH). TATTOO did not affect the levels of metals in the tissue and MT-SH. However, the rate of the metalation of MTs (MT-Me) increased only by Cd and TATTOO. Moreover, the expression of MTs detected as MT mRNA concentration was not changed in all exposures. These discrepancies in the MTs responses could be explained by some reasons: 1. shortcoming of the method of MT-SH determined after the ethanol/chloroform extraction from tissue (the extract can also contain other low weight cellular thiols); 2. imbalance in the MTs turnover. In any case, the elevation of MT-SH by exposures to metals was corresponding to the decrease in the lipid peroxidation rate, reflecting probably the scavenging activity of low weight thiols. The particular effect of TATTOO was the decrease of cholinesterase activity typical for thiocarbamates. Different manifestations of oxidative stress response were detected in each exposure. Most consecutive response was the depletion of Mn-superoxide dismutase.

P-05. Study of detoxification capabilities of fish from contaminated environment

Milena Bušová[1], Vlastimil Stejskal[2]

[1]*Institute of Hygiene and Epidemiology, First Faculty of Medicine and General University Hospital in Prague, Charles University, Prague, Czech Republic*
[2]*Institute of Aquaculture and Protection of Waters, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic*

Healthy aquaculture is dependent on the quality of the aquatic environment. The aquatic organisms living in water environment contaminated by products of human activities have to cope with the presence of contaminants. Among the most important human waste products, besides the presence of pharmaceuticals, plastics and nanoparticles of various origins, belong the heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb) and arsen (As). The chronic effect of these contaminants is dangerous on predatory and longer-lived fish. In their tissues, heavy metals can be accumulated at higher concentrations. A major problem is the pollution of the marine ecosystem due to all human activities. Marine products used for human consumption often exceed allowable limits for heavy metals in tissues. Serious problem is e.g. the presence of mercury in the tissues of fish and seafood products, reported often by the Rapid Alert System for Food and Feed (RASFF) in Europe. Especially, an organic form, the methylmercury is toxic and dangerous to human health. The rivers under the large agglomerations, or in areas with industrial pollution, can be contaminated with heavy metals and other factory pollutants and wastes from industry or agriculture. Wastewaters from households contain drug residues and their metabolites. Through the purification processes of sewage treatment plants, they will enter into surface waters and contaminated the aquaculture.
Fish are equipped with detoxification mechanisms to protect them from the toxic effects of pollutants only to a certain extent. The active detoxifying mechanism is biosynthesis of metallothioneins (MT), or reaction with reduced glutathione (GSH).
Humic acids (HA) are known as substantives with varied structures and capability to form complexes with heavy metals. Humic acids are successfully used as a feed additive in livestock breeding. HA have a positive effect on the health of animals, growth characteristics, production parameters, and overall performance. The preventive effect of HA against the accumulation of heavy metals in tissues in farm animals is reported, too. The HA also have a beneficial effect on aquaculture. The positive effects of HA on the overall health status of fish, and on the prevention of fungal fish diseases, were reported.
In our study, we focused on therapeutic and preventive effects of humic acids on freshwater fish in fish farming.

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P-06. Serum copper concentrations in dogs with skin diseases

Emilio Carpenè, Francesco Dondi, Letteria Chiarenza, Paolo Famigli Bergamini, Gloria Isani

Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna

Introduction: Copper is an essential trace element involved in several biochemical processes. In the meanwhile, free copper is toxic and it could cause oxyradicals production. Therefore, several genes encode specific proteins controlling copper homeostasis. In particular, metallothionein, which sequesters free copper and store the metal in an inactive form. It has been reported that serum copper concentrations in bullterrier with lethal acrodermatitis and in dogs with sarcoptic mange were significantly lower than controls. The aim of our work was addressed to investigate the relationships between common pathologies, which can cause skin diseases in dogs (allergic dermatitis, inflammatory bowel disease (IBD), Cushing syndrome) and serum copper concentrations together with selected biochemical blood analytes.
Material and Methods: Serum copper concentrations were analyzed by flameless atomic absorption spectrophotometry in 22 healthy controls, in 13 dogs with allergic dermatitis, in 12 dogs with IBD and in 13 with Cushing. In the same samples were also determined total proteins, albumin and globulins. Diagnosis was achieved based on clinical examination and clinicopathological, histopathological and imaging data, following standard protocols for the selected diseases.
Results and Discussion: Mean serum copper concentrations of 0.47±0.06, 0.61±0.09, 0.58±0.22 and 0.56±0.18 were determined in control dogs and in dogs with allergic dermatitis, IBD and Cushing respectively. Only dogs with allergic dermatitis presented metal concentrations significantly higher than controls ($p<0.05$). The same dogs had a significantly lower ratio albumin/globulins respect to controls ($p<0.01$). Similar results were found in dogs with zinc responsive dermatosis (Beigh et al., 2014). Higher serum copper concentrations observed in animals with allergic dermatitis could be linked to an increase in caeruloplasmin and α2-macroglobulin, copper-containing globulins involved in copper metabolism.

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P-07. Environmental exposure to lead in urban bats from Rome: a case of intoxication?

Giulia Andreani[1], Emilio Carpenè[1], Annunziata Canavacciuolo[2], Enea Ferlizza[2], Simonetta Menotta[2], Giorgio Fedrizzi[2], Gloria Isani[1]

[1]*Department of Veterinary Medical Sciences, Alma Mater Studiorum, University of Bologna*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Dipartimento Chimico, Bologna*

Intoduction: Potential toxic effects of inorganic contaminants on wild bat populations are scarcely reported in literature, however for synanthropic species, as *Tadarida teniotis* living in an urban contest, a particular attention should be paid to the risk of exposure to toxic metals as Pb resulting from human activities. The aim of this work was to determine the concentrations of ten toxic trace elements in tissues of lactant *T. teniotis* from a nursery colony located in an urban area characterized by high anthropogenic impact.
Material and Methods: Bone, liver, kidney were collected from 30 specimens of *T. teniotis* belonging to a colony located in the "Quartiere Africano" of Rome, living in a cavity between two buildings. Ten toxic trace metals (Al, As, Ba, Cd, Hg, Pb, Sb, Sr, Th, Tl) were analysed by ICP-MS after wet digestion. Metal concentrations are reported as μg/g wet weight (w.w.).
Results and Discussion: Metals were present at detectable concentrations in analysed tissues with the exception of Cd and Th. Concentrations of most of the analysed metals were significantly higher in the bone ($p<0.01$), whereas Hg, Sb and Tl were higher in the liver ($p<0.01$). Hg and As concentrations were slightly above the LOQ in most tissues, while Pb levels were present in concentrations as high as 168±53 μg/g w.w. in bone, 66±27 μg/g w.w. in liver and 5.7±2.9 μg/g w.w. in kidney and were associated with bone lesions found at necroscopy. Toxicological threshold values for Pb in tissues of bats have not been established yet, however the concentrations found in this research are higher than those reported in bats by other authors and two orders of magnitude higher than toxic threshold levels reported for waterfowl. Lead concentrations determined in the analysed specimens could be suggestive of an intoxication status.

P-08. Lead concentrations in vultures from 4 European countries

Enrico Bassi[1], Martina Betti[2], Simonetta Menotta[2], Giorgio Fedrizzi[2], Fabiana Cappi[2], Maria Ferloni[3], Roberto Facoetti[4], Alessandro Bianchi[2]

[1]*Ersaf Direzione Parco Nazionale dello Stelvio, Bormio, Sondrio, Italy*
[2]*Istituto Zooprofilattico Sperimentale L.E.R. Bologna, Sondrio, Italy*
[3]*Provincia di Sondrio, Sondrio, Italy*
[4]*UTR Brianza Lecco, Lecco, Italy*

Introduction: Very few data are available on lead levels in tissues of vultures; therefore the aim of the present work was to compare isotopic ratios of lead concentrations in the vultures carcasses with isotopic signature found in 19 types of hunting ammunition widely used in the Alps.
Material and Methods: Lead concentrations were analysed by ICP-MS in liver, kidney and bones from 49 specimens of Eurasian Griffon (*Gyps fulvus*), from 6 specimens of Black Vulture (*Aegypius monachus*) and from 5 specimens of Bearded Vulture (*Gypaetus barbatus*) recovered from 4 european countries (Italy, France, Austria and Switzerland) by Stelvio National Park and Sondrio Province since 2008.
Results and Discussion: In all the analyzed specimens lead concentrations reached the highest concentrations in bones. The maximum mean level was found in Black Vulture (13.3±25.9 mg/Kg) and the minimum in Bearded Vulture (2.7±1.5 mg/Kg). Liver and kidney mean lead concentrations were similar and lower than 1 mg/Kg, with exception of few specimens, which presented high lead concentrations; the maximum value (4.7 mg/Kg) was determined in a sample of kidney from Eurasian Griffon. Data are in the range of those reported by other authors.
In the Alps, most scavengers ingest lead from ammunition remains, by eating lived prey with embedded shots, carcasses and/or offal. Severe cases of saturnism, owing to the ingestion of high amounts of lead, caused acute poisoning in a few cases, leading to direct mortality. Sub-lethal lead quantities, however, are ingested throughout their lifetime. This intake may affect avian populations by altering cognition and behavior, diminishing reproductive success and favoring diseases, as well as starvation or traumatic events, or by increasing mortality through aggressive inter-specific interactions (Gugiatti *et al.* 2016). These evidences also demonstrated that similar recoveries are not exceptional events but more likely they represent the 'tip of the iceberg' of a substantial proportion of the Alpine populations affected by lead at sub lethal levels demonstrating the need and urgency to replace lead bullets with other not-toxic metals (mostly made in copper).

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P-09. The impact of genetic polymorphism in selenoprotein encoding genes on the markers of oxidative stress, markers of glucose and lipid metabolism and liver enzymes activity – cross-sectional study

Ewa Jablonska[1*], Sara Raimondi[2], Edyta Wieczorek[1], Magdalena Krol[1], Jolanta Gromadzinska[1], WojciechWasowicz[1], Katarzyna Socha[3], Maria H. Borawska[3], Edyta Reszka[1]

[1]*Nofer Institute of Occupational Medicine, Lodz, Poland*
[2]*European Institute of Oncology, Milan, Italy*
[3]*Medical University of Bialystok, Poland*
*Corresponding author: ewa.jablonska@imp.lodz.pl

Background: Genetic background may partially explain a great variability of the studies related to essential trace element selenium (Se) and human health. The aim of this study was to analyze the association between single nucleotide polymorphisms (SNPs) in selenoprotein encoding genes, plasma Se and markers of oxidative stress, markers of glucose and lipid metabolism as well as clinical markers of liver health.
Methods: We enrolled 508 healthy subjects from the general population of Lodz (Poland), from whom venous blood was collected to obtain erythrocytes, plasma and DNA. SNP genotyping (*GPX1* rs1050450, *SELENOP* rs3877899, *SELENOF* rs5859, *GPX4* rs713041 and *SOD2* rs4880) was performed using Taqman® SNP Genotyping Assays and High Resolution Melt Curve technique. Plasma Se concentration was determined using atomic absorption spectrometry. Oxidative stress markers included: glutathione peroxidase 1 activity (GPx1; measured with Paglia and Valentine method); superoxide dismutase activity (SOD1; measured with Beauchamp and Fridovich method); plasma concentrations of ceruloplasmin (Cp; measured with Sunderman and Nomoto method) and thiobarbituric acid-reactive substances (TBARS - marker of lipid peroxidation; spectrofluorometric method). Commercial kits were used to determine metabolic markers: fasting plasma glucose (FPG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and triglycerides (TG). The activity of liver enzymes: alanine transaminase (ALT) and aspartate transaminase (AST), were measured with kinetic spectrophotometric methods. Data were stratified according to genotypes. Genotype differences were calculated using univariate ANOVA and multivariate ANCOVA models. Covariates included: age, sex, selenium supplements, smoking and plasma Se concentration.
Results: Mean plasma Se concentration in the group was 66.2 ng/uL. *GPX1* rs1050450 polymorphism was found to be significantly associated with TBARS (p=0.01) and Cp (p=0.01). *SELENOF* rs5859 polymorphism was significantly associated with ALT and AST activities (p=0.02 and p=0.03, respectively).
Conclusions: This study suggests possible novel association between selenoprotein F and liver enzymes function.

P-10. The effect of a natural based nutritional supplement on the content of lead and some trace elements in hard tissue

Janka Vašková[1], Marek Stupák[1], Pavol Mudroň[2], Ladislav Vaško[1]

[1]*Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Tr. SNP 1, 040 66 Košice, Slovak Republic, janka.vaskova@upjs.sk*
[2]*Clinic for Ruminants, The University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic*

Lead is considered to be non-biodegradable pollutant. In the body, it exhibits cumulative effects and causes disruption of the function of many systems. In the treatment of heavy metal intoxication, chelation therapy is used, but often with adverse effects. The aim of the study was to verify properties of the humic acids (HAs) based product with regard to its ease use and availability.

In the 10 weeks lasting experiment Ro-2575/14-221 the lead was administered over 5 weeks at a daily dose of 1/30 LD50 (155.5 mg/kg body weight) in drinking water. The administration of HAs (in 0.5%, 1% and 2% concentrations) lasted for 10 weeks. Totally 210 laboratory rats were separated into 8 groups according to administration of substances and their combinations. After the first, fifth and tenth weeks, the rats were sacrificed and the content of Zn by flame atomic absorption spectroscopy, that of Cu, Mn, Se by graphite furnace atomic absorption spectrometry in left femur were determined.

After the first week, there was an increase in Se and decrease in Pb in all groups with HAs administered. Concentrations of Mn, Cu and Zn tent to decrease. On the fifth week, there were no reduced concentrations of Se and the least significant change in Mn and Zn in group administered 1% HAs were detected. Five weeks after the finishing of lead administration we have detected the lowest rate of changes or even none in comparison to the control group only in the group where 1% HAs were administered. Similarly to the results from measurements in organs and plasma, 1% HAs seem to be beneficial in the treatment of lead intoxication.

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Keywords: hard tissue; humic acids; chronic lead intoxication; microelements; redistribution

P-11. The effect of chronic lead exposure on redistribution of some trace elements in hard tissue

Marek Stupák[1], Janka Vašková[1], Pavol Mudroň[2], Ladislav Vaško[1]

[1]*Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Tr. SNP 1, 040 66 Košice, Slovak Republic, marek.stupak@upjs.sk*
[2]*Clinic for Ruminants, The University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic*

In the body lead indirectly induces oxidative stress and exhibits toxicity by competition with Ca^{2+} and Zn^{2+} for binding sites in active molecules. The lead concentrations in bones is thought to be appropriate as a cumulative dosimeter of lead exposure. The aim of this work was to compare the rate of lead deposition in rat bones with other bodies after 5-week administration of lead as well as to control the redistribution of other elements like Se, Mn, Cu and Zn.
In the 10 weeks lasting experiment on rats, approved under the number Ro-2575/14-221, the lead was administered over 5 weeks at a daily dose of 1/30 LD50 (155.5 mg/kg body weight) in drinking water. After the first, fifth and tenth weeks, the rats were sacrificed and the content of Zn by flame atomic absorption spectroscopy, that of Cu, Mn, Se by graphite furnace atomic absorption spectrometry in left femur were determined.
After the first week, there was a significant increase in Pb concentrations in the bones, but also a decrease in Se when compared to the control group. However, within organ homogenates and plasma, increased Pb concentrations were found only in plasma. Trend in bones continued until the fifth week, with a significant decrease in Mn, Cu, Zn. From other organs, increased Pb was found only in plasma and vice versa, in heart, kidney, or liver, concentrations of Mn, but also Zn and Cu were mainly increased. After the fifth week, lead was no longer served. At week 10, we found still significant increase in Pb concentrations in the bones. Se, Mn and Cu concentrations showed no change, but Zn concentrations increased. Concentration of lead in bones reflects exposure more aptly and for a longer time than other organs of deposition.
The publication of the study was supported by VEGA 1/0559/18.
Keywords: hard tissue; chronic lead intoxication; microelements; redistribution

P-12. Changes of elemental composition of erythrocytes patients with acute and chronic disorders of cerebral circulation

Aliaksandra S. Skarabahatava[1], Julia Stepanova[2], Ekaterina I. Slobozhanina[1], Ludmila M. Lukyanenko[1], L.I. Alechnovich[3]

[1]*Institute of Biophysics and Cell Engineering of NAS of Belarus*
[2]*Republican Research and Practical Center of Neurology and Neurosurgery*
[3]*Belarussian Medical Academy of Postgraduate Education*

The aim of the study was to analyze the elemental composition of erythrocytes in 29 patients with acute cerebral infarction (CI) (mean age 72.85 ± 10.11) and in 18 patients with chronic dyscirculatory encephalopathy (DEP) (mean age 79.00 ± 15.56), the blood of healthy donors was taken as a control. On the 1st day of hospitalization it was determined the content of the essential elements in isolated erythrocytes using the atomic-emission spectroscopy with inductively coupled plasma (ICPE-9000, Shimadzu, Japan).
At acute CI an increase the intra-erythrocyte content of Ca & Na and decreasing of K, Mg, Fe & Zn were found in contrast with donor erythrocytes. Patients with DEP had a deficiency of K, Fe, P & Zn in contrast with donor's cells. Decreasing of Fe & Zn in both groups in contrast with donors reflect the developed misbalance of antioxidants/prooxidants in erythrocytes under acute and chronic disorders of cerebral circulation.
The established changes of the elemental composition of erythrocytes in patients with CI and DEP indicate a disturbance of the metal-ligand homeostasis in acute and chronic disorders of cerebral circulation, which makes an undeniable contribution to the pathogenesis of ischemic cerebrovascular pathology.

P-13. Microviscosity of lipid bilayer of human erythrocytes effected by complexes amyloid structures with aluminum ions

Aliaksandra S. Skarabahatava, E.I. Venskaya

Institute of Biophysics and Cell Engineering of NAS of Belarus, Minsk

The aim of the study was to investigate changes in microviscosity of erythrocyte membranes effected by lysozyme amyloid structures in complex with aluminum ions. For analysis, we used two fluorescent dyes (TMA-DPH, laurdan); membrane lipid peroxides (LPx) were measured on the based on lipid peroxides reaction with thiobarbituric acid.

Our results confirm that human erythrocytes exposed to complexes of amyloid structures with aluminum undergo physico-chemical modifications at the membrane level which differ from influence only amyloid structures. We founded a decrease of level LPx after influence only amyloid fibrils. But in contrast complex fibrils with aluminum ions leads to increasing level of LPx. Also this complexes leads to decrease in fluorescence anisotropy of TMA-DPH and in the polarity of the lipid bilayer with a concomitant shift toward a gel phase, which reflected by increasing of general polarisation of laurdan fluorescent. Again results obtained after only amyloid fibril influence were quite the opposite.

Our results showed that membrane effects of amyloid structures in complex with aluminum ions mostly determent by aluminum ions.

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P-14. Correlation between dietary selenium exposure with biochemical and metabolic parameters: A cross-sectional study in Northern Italy population

Marco Vinceti[1,2], Daniela Lasagni[3], Francesco Bruzziches[1], Claudia Baraldi[1], Carlotta Malagoli[1], Sara Grioni[4], Sabina Sieri[4], Annalisa Santachiara[5], Tiziana De Luca[3], Thelma Pertinhez[3,6], Roberto Baricchi[3], Tommaso Filippini[1]

[1]*Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[2]*Department of Epidemiology, Boston University School of Public Health, Boston, USA*
[3]*Transfusion Medicine Unit, AUSL-IRCCS, Reggio Emilia, Italy*
[4]*Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*
[5]*AVIS – Section of Reggio Emilia, Reggio Emilia, Italy*
[6]*Department of Medicine and Surgery, University of Parma, Parma, Italy*

Background and aim: The metalloid selenium shows an intriguing role with human health, with both nutritional and toxicological effects. In particular, recent studies suggest that high selenium exposure could be associated with impairment of metabolism of lipids, glucose and thyroid function. This study aims at assessing dietary levels of exposure to selenium and assess the correlation between selenium levels and biochemical and metabolic parameters in an Italian community.
Methods: In a sample adult population of ever smokers from Reggio Emilia Province we estimated dietary selenium intake through a food frequency questionnaire, validated for the Northern Italy population. From each participant we collected a fasten blood sample for determination of biochemical parameters and hormone levels, including alanine transaminase, blood glucose, total cholesterol, high-density lipoproteins (HDL), and thyroid-stimulating hormone (TSH). All subjects who participated to this study signed a written informed consent.
Results: In participants recruited from March 2017 to May 2018 the mean (standard deviation) dietary selenium intake was 101.0 (47.3) μg/day. We found negative correlation between selenium intake and HDL levels, while a positive one with TSH levels. Moreover, in sex-stratified analysis, we found a positive association between selenium intake with blood glucose levels in females.
Discussion: The dietary selenium intake in our Italian population is far above the recommended intake of 70 μg/day by the European Food Safety Authority. Our correlation analyses suggest that even at levels below the upper toxicity limits and generally considered safe, adverse effects on endocrine and metabolic systems could not be excluded, with possible sex-related differences in toxicity susceptibility.

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P-15. Correlation between dietary cadmium exposure with biochemical and metabolic parameters: A cross-sectional study in Northern Italy population

Tommaso Filippini[1], Daniela Lasagni[2], Francesco Bruzziches[1], Claudia Baraldi[1], Marcella Malavolti[1], Sara Grioni[3], Sabina Sieri[3], Annalisa Santachiara[4], Tiziana De Luca[2], Thelma Pertinhez[2,5], Roberto Baricchi[2], Marco Vinceti[1,6]

[1]*Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[2]*Transfusion Medicine Unit, AUSL-IRCCS, Reggio Emilia, Italy*
[3]*Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy*
[4]*AVIS – Section of Reggio Emilia, Reggio Emilia, Italy*
[5]*Department of Medicine and Surgery, University of Parma, Parma, Italy*
[6]*Department of Epidemiology, Boston University School of Public Health, Boston, USA*

Background and aim: Cadmium is a heavy metal classified as carcinogen for humans. It accumulates in the organism, especially in kidney and liver. Recent findings suggested that cadmium could influence human metabolism acting as endocrine disruptor and high cadmium exposure has been associated with impairment of cardiovascular and endocrine systems. This study aims at assessing the dietary intake of cadmium in an Italian community of Northern Italy and to evaluate its correlation with endocrine and metabolic factors.
Methods: In a sample adult population of ever smokers from Reggio Emilia Province we estimated dietary cadmium intake through a food frequency questionnaire, validated for the Northern Italy population. From each participant we collected a fasten blood sample for determination of biochemical parameters and hormones levels, including alanine transaminase, blood glucose, total cholesterol, high-density lipoproteins (HDL), and thyroid-stimulating hormone (TSH). All subjects who participated to this study signed a written informed consent.
Results: We eventually recruited 104 participants (men/women: 46/58), with mean (standard deviation) dietary intake of cadmium of 16.0 (8.5) μg/day. Correlation between cadmium intake and biochemical factors demonstrated a positive association with total cholesterol levels, blood glucose and TSH. Adjustment for main confounders, including sex, age, and bass index did not substantially alter the results. No clear correlation emerged with other parameters under investigation.
Discussion: The results show that in our sampled population, dietary intake of cadmium is similar with other Italian and European populations. They also suggest that cadmium intake could influence the levels of metabolic and other biochemical factor which are important risk factors for chronic cardiovascular and endocrine system diseases.

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P-16. Food contamination from the food packaging metals aluminum and tin: estimation of their dietary exposure in an Italian adult community

Tommaso Filippini[1], Stefano Tancredi[1], Carlotta Malagoli[1], Silvia Cilloni[1], Marcella Malavolti[1], Federica Violi[1], Luciano Vescovi[1], Annalisa Bargellini[1], Marco Vinceti[1,2]

[1]Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[2]Department of Epidemiology, Boston University School of Public Health, Boston, USA

Background and aim: Aluminum and tin are metals widely used by humans as food packaging material and in the general population the dietary intake it one of the most common source of exposure. The concentration in foods is variable and depends on the original food content and to through contamination from food packaging containers and cookware. High metal exposure and in particular to aluminum has been suggested to play a role in the neuronal toxicity leading to Alzheimer's Dementia. This study aims at estimating the aluminum and tin dietary intake of an Italian adult population.
Methods: Food consumption data were retrieved through administration of a validated semi-quantitative food frequency questionnaire in a representative sample population of Northern Italy. We collected a pooled sample of food composing their diet in order to measure aluminum and tin concentration by inductively coupled plasma mass spectrometry and we eventually estimated their dietary intake combining dietary habits with metal measurements in food.
Results: We collected a pooled sample of 908 foods. The highest levels were found for aluminum in sweets, cereals and vegetables, and for tin in sweets, meats, fish and seafood. The median estimated daily dietary intake of aluminum was 6133.5 μg/day (Interquartile range - IQR: 3903.3 - 18.231.1 μg/day), with major contribution from beverages and vegetables, followed by cereals. For tin, we estimated a median intake of 68.1 μg/day (IQR: 47.7-94.5 μg/day) with major contribution from vegetables and fruits, followed by meat and dairy products.
Discussion: Our results provide an estimation of dietary intake of aluminum and tin in an Italian community of Northern Italy, since the estimation are based on metals measurements in foods actually consumed by the population under investigation. Finally, the use of a validated food frequency questionnaire in assessing food consumption pattern data improved the accuracy of our updated results.

P-17. The new-old exposure to lead: assessment of food contamination and estimation of dietary intake in a Northern Italy population

Tommaso Filippini[1], Marcella Malavolti[1], Silvia Cilloni[1], Federica Violi[1], Carlotta Malagoli[1], Luciano Vescovi[1], Marco Vinceti[1,2]

[1]*Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[2]*Department of Epidemiology, Boston University School of Public Health, Boston, USA*

Background and aim: Lead is a heavy metal released in the environment after natural and anthropogenic activities. Food and water are the major sources of human exposure, with some through air, dust and soil. In this study, we aimed at characterizing lead content in foods consumed in Northern Italy population in order to estimate its dietary intake.
Methods: To do that, we collected food samples during the period from October 2016 to February 2017 from local markets, and we measured elements content using inductively coupled plasma-mass spectrometry. Finally, we assessed dietary habits of a Northern Italian community though a validated food frequency questionnaire, and we eventually estimated dietary intake of lead in that community.
Results: In the 908 analyzed food samples, highest lead contamination levels were found in seafood, vegetables, sweets and beverages. The estimated dietary intake was 12.94 μg/day (interquartile range-IQR 9.36-17.51 μg/day), corresponding to 0.186 (IQR 0.133-0.454) μg/kg of body weight/day, with major contribution from beverages and vegetables and cereals. Similar results were found in both sexes, with daily dietary intake of 14.13 μg/day (IQR 10.10-17.82) and 12.19 μg/day (IQR 8.97-17.36) in men and women, respectively.
Discussion: Our study provides an updated assessment of lead exposure through diet in a Northern Italian community. Although environmental lead contamination and exposure has markedly decreased in the last decades, recent findings pointed out that a safety threshold value could not be established for prevention of adverse effects due to lead exposure. In our community, despite the generally low levels of intake compared with other European populations, the levels of lead intake are at still at risk for chronic renal disease and hypertension.

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