POSTER SESSION B

SelenoDB 2.0: HIGH-QUALITY ANNOTATION OF SELENOPROTEIN GENES IN EUKARYOTES AND THEIR GENETIC DIVERSITY IN HUMANS

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SelenoDB started out as a long-term project with an aim to provide, through the collaborative effort of experimental and computational researchers, high-quality annotations of selenoprotein genes, proteins and SECIS elements (Castellano *et al*. *Nucl. Acids Res*., 2008). The first release of the database included an initial set of eukaryotic genomic annotations, with special emphasis on the human selenoproteome. Since the release of SelenoDB 1.0 many new eukaryotic genomes have been sequenced, including those of the great apes. The annotation of selenoprotein genes in these genomes is usually wrong in major databases. For this reason, we have now fully annotated selenoprotein genes in 66 eukaryotic genomes (in contrast to six in SelenoDB 1.0). We used Selenoprofiles (Mariotti and Guigó, *Bioinformatics*, 2010), an automatic annotation pipeline, to annotate selenoprotein genes in these many genomes. In addition, we have surveyed genetic variation in genes that incorporate selenium (selenoproteins) or are involved in its metabolism in primates. We used exon capture and resequencing approaches to identify single nucleotide polymorphisms (SNPs) in these genes in humans, chimpanzees and bonobos. More than 900 hundred individuals from 52 human populations around the world (Africa, Middle East, Europe, Asia, Oceania and America) were surveyed. We identified thousands of SNPs in selenium related genes in these human populations. We provide, for the first time, a detailed view of the genetic diversity and divergence of selenoprotein genes in primates and eukaryotes, respectively. The addition of these large datasets into the second release of the database, SelenoDB 2.0, provides a valuable resource for addressing medical and evolutionary questions. In particular, to understand the role of natural selection in shaping the evolution of selenoprotein genes in primates and eukaryotes alike. SelenoDB is freely available at http://www.selenodb.org.

AUTOIMMUNITY AGAINST SELENOPROTEIN P IN HUMAN SERA

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Selenoprotein P (SePP) has been shown to be essential for Se homeostasis in the CNS, for Se transport into kidney and for Se reuptake from the primary urinary filtrate. These processes are mediated by SePP receptors, i.e., by APOER2 in brain and Megalin in kidney. Autoantibodies (aAB) against Megalin have been detected in a number of autoimmune diseases (AID) including rheumatoid arthritis, systemic lupus erythematosus, osteoarthritis, and Behçet's disease (1). In view of the increasing prevalence of AID, we hypothesized that also SePP may be a target of aAB. Recombinant expression of human SePP in baculuvirus-infected insect cells was achieved after replacing the Sec residues by Cys. Recombinant Sec-free SePP was isolated using chromatography on Ni-NTA agarose. The isolated proteins displayed a molecular mass heterogeneity (45-50 kDa) potentially caused by differences in glycosylation. Recombinant SePP specifically interacted with its membrane receptors on HeLa, HepG2, Raw 264.7 and MC3T3 cells, indicating its similarity to native SePP. Recombinant SePP was labeled by MACN-acridinium-NHS-ester and used to screen 180 human sera for aAB against SePP by immunoprecipitation analysis. The SePP-immune complexes were precipitated with protein A-sepharose. Pellets were washed and measured in a Berthold luminometer. Approximately 2-3% of the sera showed clear signals for SePP aAB. The physiological role of these SePP-specific aAB for SePP-mediated Se transport need to be investigated in future studies. Especially patients with AID may need to be analyzed for aAB against SePP and its receptors in order to better care for their Se status.

References:

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SELENOPHOSPHATE SYNTHETASE 1 REGULATES VITAMIN B6 METABOLISM IN DROSOPHILA: PREDICTION AND CONFIRMATION

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Selenophosphate synthetase 1 (SPS1), one of the two selenophosphate synthetases (SPS1 and SPS2) in higher eukaryotes, plays an essential role in the cell during embryogenesis and cell growth. However, the molecular pathway regulated by SPS1 is yet to be determined. In this study, we identified SPS1 regulated pathway by analysing transcriptome and gene ontology after SPS1 knockdown. Differentially expressed genes (DEGs) were identified at day1, day3 and day5 using microarray analysis after SPS1 knockdown in Drosophila SL2 cells. The DEGs were clustered according to their temporal expression pattern with SOM clustering method. Early- and late-gene sets were obtained by selecting the clusters showing significant changes in gene expression at early and late stage, respectively. To predict a biological pathway regulated by SPS1 knockdown, gene ontology analysis was performed against each gene set using BinGO software. Gene ontology terms related to vitamin B6 biosynthesis were found to be significantly affected at the early stage at which megamitochondria, the major phenotypic change observed by SPS1 knockdown in Drosophila SL2 cell, were not formed (day 3) and genes related to defense and amino acid metabolism were affected at the later stage (day 5). DEGs involved in vitamin B6 biosynthesis were down-regulated and intracellular levels of pyridoxal phosphate, an active form of vitamin B6, were decreased by SPS1 knockdown. Treatment of SL2 cells with an inhibitor of pyridoxal phosphate synthesis showed similar expression pattern with SPS1 knockdown and led to megamitochondrial formation. These results indicate that SPS1 primarily regulates vitamin B6 synthesis, which in turn impacts various cellular systems such as amino acid metabolism, defense and other important metabolic activities and finally regulates cell growth.

STUDY OF SELENIUM SUPPLY MECHANISM OF SELENOPROTEIN P USING ^{75}Se AND IMMUNOLOGICAL METHODS

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Selenoprotein P (SeP) is a selenium-rich extracellular glycoprotein and is the major selenoprotein in plasma. SeP plays an important role in the maintenance of selenium levels in the periphery tissues. It has been reported that SeP supplies selenium via lipoprotein receptors such as apolipoprotein E receptor-2; however, details of molecular mechanism are still unknown. In the present study using ^{75}Se-labeled SeP and monoclonal antibodies against human SeP, selenium-supply mechanism of SeP was investigated. The ^{75}Se-labeled SeP was prepared by human hepatoblastoma HepG2 cells. In human T-lymphoma Jurkat cells, specific binding of ^{75}Se-labeled SeP was observed with high affinity. Cross-linking study was performed to define the surface molecule on Jurkat cells responsible for SeP binding. Specific complex of 250 kDa was formed by the addition of chemical cross-linker into the cells treated with SeP. It was confirmed that this complex contained SeP protein by western blot analysis using anti-SeP monoclonal antibody. Since immunoprecipitation of this complex was succeeded, LC-MS/MS analysis of precipitants is now proceeded to identify SeP binding proteins on the cell surface. Binding of SeP to the cell surface was also detected by flowcytometer analysis using a monoclonal antibody against human SeP. It was found that some antibodies against SeP could supress both binding of SeP to the cell surface and selenium-supply activity of SeP. We identified the epitope of these inhibitory antibodies and will discuss the important site of SeP for the interaction with cells.

ESTABLISHMENT AND CHARACTERIZATION OF A NEW ELISA FOR SELENOPROTEIN P

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The accurate quantification of selenoprotein P (SePP) is of growing interest for basic research and clinical studies in a variety of areas. Currently, there is some discrepancy on SePP concentrations in humans. Two major reasons contribute to this inconsistency; the characterization and validation of some (even commercial) SePP assays is marginal or missing and there is no uniform reference material for standardization.Out of the need to compare clinical results across research groups we decided to develop a monoclonal antibody-based enzyme immunoassay according to highest standards of laboratory-developed molecular assays suitable for 96-well analysis. The assay procedure is optimized to follow a standard enzyme-linked immunoassay protocol and uses a chromogenic detection method available in most laboratories. The assay standard curve is calibrated against NIST SRM 1950 standard reference plasma. Multi-laboratory validation tests were performed according to international guidelines. The accuracy was measured with an average deviation from the true concentration of +2.9% RE. Precision was determined at three levels: repeatability (with-in plate variation) with 6.2% CV, intermediate precision (within-laboratories variation) with 10.5% CV, and reproducibility (between-laboratories variation) with 11.3% CV. The limit of quantitation was 8.0 µg/l, i.e., around 500-fold below serum SePP concentrations of well-supplied subjects. The signals were linear on dilution within the working range of the assay and SePP was stable in serum for 24 hours at room temperature.The analytical performance characteristics of this ELISA indicate that it is suitable to provide comparable results for multi-laboratory studies with clinical samples. We have thus decided to make this assay commercially available in order to support research on Se and SePP status across the different research and clinical disciplines.

BLOOM AND GLOOM: SELENOPROTEOMES OF RED AND BROWN TIDES

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Selenoproteins play diverse roles in organisms. The number of selenoprotein genes is especially high in the genomes of various aquatic species. We hypothesized that new families of eukaryotic selenoproteins are more likely to be discovered in these organisms and, following intial screening of available genomes, focused on organisms with exceptionally high selenoprotein gene content. These include a single-celled phytoplankton *Emiliania huxleyi*, known to occur everywhere except the polar regions; non-motile pelagophyte *Aureococcus anophagefferens* from northeast and mid-Atlantic US estuaries; and diatoms *Pseudo-nitzschia* from Fisheries Oceans, Canada, known to produce the neurotoxin domoic acid, a toxin responsible for the human illness calledamnesic shellfish poisoning. These species are also of special interest, because they are known to cause destructive "red tide" and "brown tide" blooms. The *Aureococcus* genome was the first genome sequenced of any Harmful Algal Bloom species, and we found that this organism has the largest and the most diverse selenoproteome identified to date. It consists of at least 59 selenoproteins, including known eukaryotic selenoproteins, selenoproteins previously only detected in bacteria, and novel selenoproteins. Oxidoreductase functions were assigned to the majority of detected selenoproteins, and we found that the insertion of Sec in these proteins was supported by a unique Sec insertion sequence. Significantly overlapping, but not identical sets of selenoprotein genes were discovered in other algal genomes. Our findings highlight the need for careful analysis of selenocysteine-containing genes with non-canonical SECIS elements. This analysis also indicates that numerous additional selenoprotein families are yet to be discovered.

FUNCTIONAL ADAPTATIONS TO ENVIRONMENTAL SELENIUM IN VERTEBRATE EVOLUTION

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Over the past 530 million years, the vertebrate lineage has evolved from primitive jawless fish to all the diverse forms of fish, amphibians, reptiles, birds and mammals we see today. These vertebrate species have come to inhabit most of the earth environments. These environments differ widely in their selenium levels in the water or soil and this, coupled with the distinct diet of each species, has led to a wide range of selenium intake levels in vertebrates around the world. Shifts in dietary selenium intake sustained over many generations are a likely selective pressure in vertebrates.Interestingly, there is great variation in the number of selenoprotein genes across vertebrate species. For example, humans have 25 selenoprotein genes while zebrafish has 39. Similarly, the number of genes in the different selenoprotein gene families (*e.g.* GPx) also varies widely across vertebrate species. Moreover, the number of Sec residue in Selenoprotein P of different vertebrate species also varies in a wide range (10 in Humans and 17 in Zebrafish). Thus, vertebrate species are likely to have different levels of dependence on the trace element selenium.Such distinct reliance on selenium raises the question of whether natural selection has shaped the use of this micronutrient throughout vertebrate evolution. To test this hypothesis, we look for signatures of functional adaptations in the coding regions of selenoprotein genes in a large number of vertebrate species. In particular, we look for an excess of amino acid substitutions between (orthologous genes) and within (paralogous genes) vertebrate species. We statistically test whether this excess of amino acid changes is expected under neutral evolution. This approach allows us to identify amino acid sites, selenoprotein genes and vertebrate lineages that may harbor adaptations to the levels of selenium in the environment.

THE ENZYMATIC ACTIVITY OF SELENOPROTEIN S

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Among the 25 human selenoproteins, Selenoprotein S (SelS, VIMP) and Selenoprotein K (SelK) are unique in that they are intrinsically disordered proteins. SelS is a member of the endoplasmic reticulum associated protein degradation pathway - a quality control system responsible for dislocation of misfolded proteins from the ER for degradation in the cytoplasm. We establish that SelS is a reductase and that thioredoxin acts as its electron donor. In addition to its ability to reduce disulfide bonds, SelS is capable of reducing hydrogen peroxidase. However, it is not a broad substrate peroxidase. The peroxidase reaction includes the formation of the reaction intermediate, selenenic acid (SeOH), which can be trapped when the resolving cysteine is mutated. To further investigate the unique role of selenocysteine, we compare the wild type SelS to the cysteine-containing mutant. The cysteine-mutant is not enzymatically active even though its redox potential is only 23 mV lower than that of the wild type. In addition, SelS can rapidly reform its selenenylsulfide bond following its reduction, and it can resist inactivation by H_2O_2. These features allow SelS to both detect and evade damage by reactive oxygen species.

THE ROLE OF GLUTATHIONE PEROXIDASE 2 DURING COLITIS

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The selenoprotein glutathione peroxidase 2 (GPx2) is specifically located in the gastrointestinal epithelium and is further up-regulated in patients suffering from ulcerative colitis. In cell culture experiments, GPx2 inhibits IL-1β-induced cyclo-oxygenase 2 (COX-2) expression. GPx2-knockout (GPx2-KO) mice develop a more severe intestinal inflammation than wildtype (WT) mice after treatment with azoxymethane and dextran sodium sulfate (DSS). Based on these results, GPx2 appears to have anti-inflammatory properties. To further elucidate the role of GPx2 in colitis, we aimed to analyse its expression and transcriptional regulation in cytokine-treated cell culture and during DSS-colitis in mice. In the colon of DSS-treated mice, GPx2 was up-regulated especially in regenerative crypts next to ulcerations. In cell culture, GPx2 expression and promoter activity was unaffected by classical pro-inflammatory mediators like PGE_2, TNF-α, and IL-1β while it was up-regulated by the anti-inflammatory mediators 15d-PGJ_2 and IL-22. 15d-PGJ_2 is a well-known activator of the Nrf2 system. Thus, it was expected to activate the promoter of the Nrf2 target gene GPx2. The mutation of the antioxidant responsive element (ARE) within the GPx2 promoter abolished the stimulatory effect of 15d-PGJ_2. IL-22 is known to activate transcription factors of the Stat family. Therefore, we analysed the GPx2 promoter for putative Stat-responsive elements. Point mutation of the binding element next to the transcription start completely abolished promoter activation after IL-22 treatment, indicating that GPx2 is a new target gene of Stat transcription factors. In summary, 15d-PGJ_2 and IL-22 both

have anti-inflammatory properties and support wound healing in the injured intestinal epithelium. The up-regulation of GPx2 by both mediators can explain its specific localization in regenerating crypts and indicates that GPx2 might be involved in the resolution of inflammation.

STOICHIOMETRY OF THE TERMINAL CATALYTIC COMPLEX IN SELENOCYSTEINE SYNTHESIS

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Selenocysteine, the 21st amino acid, is synthesized from a serine precursor in a series of reactions that require selenocysteine tRNA ($tRNA^{Sec}$). In archaea and eukaryotes, *O*-phosphoseryl-$tRNA^{Sec}$:selenocystenyl-$tRNA^{Sec}$ synthase (SepSecS) catalyzes the terminal synthetic reaction of selenocysteine during which the phosphoseryl intermediate is converted into the selenocysteinyl moiety while attached to $tRNA^{Sec}$. This reaction also requires a pyridoxal phosphate cofactor and selenophosphate. We have previously shown that only the SepSecS tetramer is capable of recognizing the distinct fold of $tRNA^{Sec}$. The crystal structure of the binary complex revealed that, in spite of containing four tRNA-binding sites and active-site grooves, the enzyme simultaneously bound only two tRNA molecules. Because the crystal contacts hindered substrate binding to the remaining sites, a question was raised if the observed arrangement was a consequence of the crystal packing and that it did not represent a biologically significant ribonucleoprotein complex. Herein, we determined the exact stoichiometry of the human terminal synthetic complex of selenocysteine in solution by using small-angle X-ray scattering, multi-angle light scattering, and analytical ultracentrifugation. We unambiguously show that SepSecS preferably binds one or two $tRNA^{Sec}$ molecules at a time. Intriguingly, when two tRNAs are bound, one homodimer always serves as a docking platform, whereas the other one plays a role as the catalytic unit. Our results not only confirm the architecture previously observed in the crystal, but also suggest that allosteric regulation of SepSecS might play an important role in synthesis of selenocysteine and selenoproteins.

A REGULATORY FUNCTION OF SELENOPROTEIN W

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Selenoprotein W (SelW) contains a selenocysteine (Sec; U) in the CXXU motif corresponding to the CXXC motif in thioredoxin (Trx) and thus it appears to be involved in regulating the cellular redox state. However, the precise function of SelW has not yet been elucidated. We found the SelW was interacted with 14-3-3, and some cellular events were controlled by the interaction of the proteins. Thus, SelW was involved in the G2-M transition, especially in the recovery from G2 arrest after DNA damage. Knockdown of SelW significantly accumulated phosphorylated Cdk1, which eventually led to a delay in recovery from G2 arrest. The inactive Cdk1 was caused by the sustained inactivation of CDC25B, which removed the inhibitory phosphate from Cdk1. Our observation from this study reveals that SelW activated CDC25B by promoting the dissociation of 14-3-3 from CDC25B through the reduction of the intramolecular disulfide bond during recovery. And we also found that SelW was involved in cell proliferation by regulating the activity of Akt kinase. Activation of Akt is enhanced by activating the mammalian target of rapamycin complex 2 (mTORC2). 14-3-3 is also a negative regulator of the mTORC2/Akt pathway by interacting with a component of mTORC2. In this study, we found that the binding of Rictor, a component of mTORC2, to 14-3-3 was regulated by the interaction of 14-3-3 with SelW. When SelW was down-regulated, mTORC2-dependent phosphorylation of Akt at Ser473 was decreased. However, the phosphorylation of Thr308 was not affected. Taken together, we suggest that SelW is involved in cellular regulation through interacting with 14-3-3.

EXPRESSION PROFILE OF SELENOGEMONE IN LIVER AND MUSCLE OF CHICKS PRIOR TO THE ONSET OF EXUDATIVE DIATHESIS

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Exudative diathesis (ED) is one of the classical diseases induced by dietary Se and vitamin E deficiencies in chicks. To reveal mechanisms for ED, we fed 4 groups of day-old broiler chicks (n = 60/group) with a corn-soy basal diet (BD; 14 μg Se/kg; no vitamin E added), the BD plus all-rac-a-tocopheryl acetate at 50 mg/kg, Se (as sodium selenite) at 0.3 mg/kg, or the two nutrients for 6 wk. High incidences

of ED (60%) and mortality (35%) of chicks were induced by BD, starting at wk 3. Because both were completely prevented by dietary Se supplementation, we determined mRNA levels of 14 selenoprotein genes in liver and muscle of chicks at wk 6. Using samples collected from the same experiment, the present study was followed to elucidate how gene expression of 22 selenoproteins in liver and muscle of chicks responded to dietary Se and vitamin E deficiencies at wk 2 prior to the onset of ED. Dietary Se deficiency decreased ($P < 0.05$) mRNA levels of Gpx1, Gpx3, Gpx4, Sepw1, Sepp1, Selo, Selk, Sep15, Selu, and Selh in both muscle and liver. It also downregulated ($P < 0.05$) mRNA levels of Sepn1, Selt, and Dio1 in liver. Despite 6 common genes (Gpx1, Gpx4, Sepw1, Sepp1, Selo, and Selk) suppressed by dietary Se deficiency in liver and muscle at wk 2 and wk 6, responses of muscle Sepn1 and liver Seli mRNA levels were different between these time-points. Dietary vitamin E deficiency upregulated ($P < 0.05$) muscle Gpx7 and Sepw1 and hepatic Sels, Seli, Sepn1, Txnrd1, and Txnrd2 mRNA levels at wk 2.

There were interactions ($P < 0.05$) between dietary Se and vitamin E on hepatic Sepn1 and muscle Sepw1 and Sep15 mRNA levels. In conclusion, responses of selenogenome expression in liver and muscle of chicks to dietary Se and vitamin E deficiencies were not all the same between before and after the onset of ED. These differences may offer us new clue on the role of selenoproteins in the pathogenesis of ED.

ROLE OF SelR IN CYTOPROTECTION AGAINST GALACTOSE-INDUCED APOPTOSIS OF HUMAN LENS EPITHELIAL CELLS

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Cataract is one of the major complications of diabetes mellitus. Oxidative stress is believed to play a major role in cataract formation. The galactose cataract model has been widely used in investigating the effect of various stresses on eye lens, exploring various mechanisms of cataract formation, as well as screening for anti-cataract drugs. Selenoprotein R (SelR) named methionine sulfoxide reductases B1 (MsrB1) is localized in the cell nucleus and cytosol. Previous studies and our group demonstrated that SelR in lens cells is important for the maintenance of lens cell viability and resistance to oxidative stress damage. In order to investigate the roles of SelR in cytoprotection against diabetic cataract, we studied the influences of SelR gene silencing on galactose-induced apoptosis in human lens epithelial cells (hELCs) which were cultured in DMEM-10% FBS medium and divided four groups: normal, galactose (150mM) exposed, SelR-gene-silenced cells and SelR-gene-silenced cells exposed galactose. In all cases we detected cell viability, cell apoptosis rate, intracellular reactive oxygen species (ROS) and malondialdehyde (MDA) levels, alteration of mitochondrial membrane potentia as well as the activity of caspase-3. The results showed that the cell viability was decreased with the increase of galactose dose, both exogenous galactose and SelR gene silencing by siRNA independently resulted in oxidative stress, elevated the levels of ROS and MDA, decreased $\Delta\psi m$ as well as increased activity of caspase-3 and the percentage of apoptotic cells. When SelR-gene-silenced cells were exposed to galactose, these indexes were further aggravated at the same conditions. These results suggest that SelR plays important roles in protecting hLECs against galactose-induced oxidative damage and inhibiting oxidative stress-induced apoptosis, which is an early event known to contribute to cataract formation.

SELENOPROTEIN W GENE SILENCING INFLUENCE INTRACELLULAR CALCIUM ION LEVEL AND THE EXPRESSION OF ENDOPLASMIC RETICULUM RESIDENT SELENOPROTEINS IN MYOBLAST OF CHICKEN

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Selenoprotein W (SelW) expresses particular high in muscle tissues, which plays an important role in the metabolism of these tissues. Many studies focused on the function of its anti-oxidative property, however little was known about its role in calcium regulation in chicken myoblast. To examine its calcium homeostasis regulation function in chicken myoblast, we silenced the expression of SelW by using the small interfering RNAs (siRNAs) technology and found that the SelW deficiency influenced the normal intracellular calcium ion ($[Ca^{2+}]i$) level suggesting that SelW may influence calcium homeostasis of chicken myoblast. Furthermore, we found that the loss of SelW influenced the mRNA expression of some endoplasmic reticulum resident selenoproteins, selenoprotein T (SelT), selenoprotein S (SelS), selenoprotein K (SelK), selenoprotein N (SelN) that involved in the regulation of $[Ca^{2+}]i$. These results demonstrated that SelW played a role in the regulation of $[Ca^{2+}]i$ of chicken myoblast. And some endoplasmic reticulum resident selenoproteins may functionally compensate for SelW.

LOCALIZATION AND REGULATION OF PANCREATIC SELENOPROTEIN P

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Progressive loss of pancreatic beta-cell mass is a crucial feature of type 2 diabetes mellitus. As beta-cells express very low amounts of the antioxidant enzymes catalase and glutathione peroxidase (GPx), they appear to be particularly vulnerable to oxidative damage in the pathogenesis of diabetes. We investigated the pancreatic expression pattern and regulation of selenoprotein P (Sepp1) that may serve as antioxidant enzyme in addition to its main function as selenium (Se) transport protein. Sepp1 was detected in rodent pancreas by immunofluorescence and real-time RT-PCR. Regulation of Sepp1 biosynthesis in INS-1 rat insulinoma cells was investigated by real-time RT-PCR, luciferase gene reporter assay and immunoblotting. Sepp1 and GPx1 gene expression in rat pancreas were 58% and 22%, respectively, of the liver values. Pancreatic Sepp1 expression was restricted to the endocrine tissue, being present in alpha- and beta-cells of mouse islets. In INS-1 insulinoma cells, Sepp1 biosynthesis was stimulated by the selenium compound sodium selenate and diminished in the presence of high glucose (16.7 vs. 5 mM) concentrations. Sepp1 mRNA stability in INS-1 cells was also lowered at 16.7 mM glucose. Moreover, Sepp1 mRNA levels were decreased in isolated murine islets cultured in high glucose (22 mM) medium compared to normal glucose (5.5 mM). Pancreatic Sepp1 expression was elevated upon treatment of mice with the beta-cell toxin streptozotocin.

Conclusion: Pancreatic islets express selenoprotein P that may fulfil a function in antioxidant protection of beta-cells. Down-regulation of Sepp1 expression by high glucose might thus contribute to glucotoxicity in beta-cells.

SELENO-TRANSFERRIN FOR TREATMENT OF LEUKEMIA

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Background: Human leukemia is a malignancy of hematopoietic stem cells with impaired differentiation, and uncontrolled proliferation. Many leukemia cell lines over-express the membrane Transferrin receptor (TfR). Over-expression of the TfR has permitted Transferrin (Tf) to be used to target various drugs to Leukemia cells.Selenium selenides (RSe^-) generate free radicals, i.e.; superoxide (O_2^-), by redox cycling following endocytosis within cells. O_2^- generation by Se is reported to cause cell apoptosis due to increased oxidative stress. Targeting the TfR, we have covalently attached redox selenides to Apo-, Holo-Tf and Human Serum Albumin (HSA) and treated K562 and THP-1 leukemia cells.

Methods: Control K562 and THP-1 leukemia cell lines were treated in culture with Apo-, Holo-Tf and HSA covalently attached Se. Se was measured by ICP-MS. The cytotoxic effects of Se-Tfs and Se-HSA in K562 and THP-1 were analyzed at concentrations of 0.6 to 4.8 µg Se/µg of protein and incubated with cells for 24, 48, 72, 96 and 120 hours post-treatment. Cell proliferation, viability, and assessment of apoptosis were determined by visual microscopy, Trypan Blue (TB) exclusion, TB spectrophotometry, by the MTT assay for cell viability and by Caspase-3 for apoptosis.

Results: In 72 to 120 hr assays, Se-Tfs and Se-HSA treatments of both K562 and THP-1 cells in *vitro* reduced cell proliferation. At higher Se concentrations and longer time periods cell proliferation was reduced 50% and 95% compared to controls. Caspase-3 activity absorbance was increased 2 fold in the Se treated cells indicating cell death was apoptotic.

Conclusions: Se-Tfs and Se-HSA diminished cancer cell proliferation by apoptosis in comparison to controls at the same concentrations. The end purpose of this research is to develop a targeting Se-Tf-drug (a pseudo-monoclonal antibody) to treat human leukemia cells *in vivo* over-expressing Transferrin receptors.

SELENIUM SUPPLEMENTATION PROTECTS NORMAL HUMAN ESOPHAGEAL CELLS AGAINST X-RAY IRRADIATION

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Background: Although radiotherapy is effective in killing cancer cells, reactive oxygen species produced during radiotherapy may threaten the integrity and survival of the surrounding normal cells. The administration of radioprotective agents, which scavenges radiation-induced radicals and reduces effects of radiation at an early stage, has been suggested as one of approaches for post-exposure prophylaxis of radiation side effects in normal tissue. Selenium supplementation is a suggested preventive approach for radical detoxification.

Objective: The aim of this study was to investigate the protective effect of sodium selenite supplementation to normal human esophageal cell line (CHEK-1 cells) treated with X-ray irradiation.

Methods and Results: GPx activity increased dose-dependent manner and reached plateau at 50nM sodium selenite supplementation for 72h. Cell IC_{50} of sodium selenite to CHEK-1 cells was determined to be 3.6μM.

Colony formation increased in the cells supplemented with sodium selenite 25nM, 50nM, 100nM and 200nM for 72h before irradiation compared to the cells treated by 2Gy X-ray irradiation alone.

Cell viability was significantly higher ($p< 0.05$) for the cells incubated with 50nM sodium selenite for 72h before 2Gy X-ray irradiation at 72h post-irradiation compared to the cells treated by 2Gy X-ray irradiation alone.

Conclusion: The results suggest that sodium selenite supplementation at safe dose is a promising protection for prophylaxis of radiation side effects to normal cells during radiotherapy.

SELENOPROTEIN GENE EXPRESSION IS AFFECTED BY INTERACTIONS BETWEEN DIETARY SELENIUM AND METHYL MERCURY

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Elevated dietary levels of selenium (Se) can reduce methyl mercury (MeHg) toxicity, but the mechanisms behind this interaction are unclear. We explored selenoprotein gene regulation in relation to maternally transferred dietary Se (as selenomethionine) and MeHg in zebrafish (Danio rerio) embryos. Female adult zebrafish were exposed to dietary MeHg (12 mg Hg/kg) and/or selenium (10 mg Se/kg). Fertilized embryos from these females were analysed by real-time PCR for selenoprotein mRNA levels at 2 days post fertilization (dpf), and embryo locomotor activity was assessed at 3-5 dpf.The response of selenoprotein mRNA expression to MeHg and Se levels could be classed into one of three groups. The first group of genes did not respond to either MeHg or Se, including some involved in thyroid hormone metabolism (dio2), Se transport/storage (sepp1a, 1b), redox signaling (txnrd3), Sec synthesis (sps2a), or are uncharacterized (selt1a). The second group of genes were downregulated by MeHg but not affected by Se. This group included antioxidants (gpx1b), and statistical trends indicate it includes several other genes (selh; p=0.056, dio1; p=0.080, txnrd1, p=0.086). In the last group, downregulation by MeHg was prevented by additional Se, and included gpx1a and 4a. Locomotor activity of the embryos was reduced (hypoactivity) by MeHg and partially reversed by additional Se. This indicates that MeHg only downregulates a subset of the selenoprotein genes. Se only prevented MeHg downregulation for two selenoprotein genes, and Se was only able to partially reverse MeHg induced hypoactivity. Interestingly the affect of MeHg on genes was not specific to any one functional group, while the preventative effects of Se were specific to genes encoding antioxidant proteins. This suggests that Se may prevent MeHg via maintenance of cellular redox balance, but may be less able to prevent MeHg induced gene regulation in other biologically important pathways such as thyroid hormone metabolism.

APOPTOSIS AND ROS STATUS IN HUMAN MDA-MB-231 BREAST CANCER CELLS TREATMENT WITH SELENIUM COMPOUNDS

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Background: Essential trace element selenium (Se) processes anti-neoplastic action and chemo preventive properties. Recent studies have shown that organic Se compounds such as methylselenocysteine (MSC) and methylseleninic acid (MSA) may induce apoptosis on several types of cancer cells, which is associated with excessive reactive oxygen species (ROS) production. We aimed to compare the effects of Se yeast (SeY), MSC, and MSA on the anti- proliferation and ROS production of MDA-MB-231 breast cancer cell line.

Design and Methods: The cultured cancer cells were assigned to CNL (0 ng Se/ml), SeY100 (100 ng Se/ml), SeY750 (750 ng Se/ml), SeY1500 (1500 ng Se/ml), MSC1500 (1500 ng Se/ml), and MSA1500 (1500 ng Se/ml), respectively. The early and progressive apoptosis, mitochondrial membrane potential, and ROS production were measured for the intervals starting *at* 0, 6, 12, 24, 48, 72, and 96 hours.

Results: For MSA treatment, highest Se uptake was observed compared to SeY and MSC treatments. The *highest* degree of *inhibition* of cell viability and apoptosis were also in MSA treatment. SeY can cause a time- and dose-dependent reduction of viability, increase in apoptotic cells, and decline in mitochondrial membrane potential. Furthermore, treatment of MSA significantly increases the maximum progressive apoptosis and ROS production at 6 hours after incubation; however, the SeY-induced early apoptosis and ROS production were found at 48 hours, respectively.

Conclusions: SeY treatment can inhibit MD-MB-231 cell viability by the action of lower ROS-induced mitochondrial apoptosis pathway.

A PHOTOGRAPHIC COMPARISON OF SELENO-TRASTUZUMAB, TRASTUZUMAB, AND SELENITE ON INHIBITION OF THE Her2+ HUMAN BREAST CANCER CELL LINE BT-474

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Aim: To compare the cytotoxic effects of Seleno-Trastuzumab (Se-Trastuzumab), Trastuzumab and selenite on the Her2+ BT-474 breast cancer cell line.

Background: Breast cancer is the second leading type of cancer causing mortality among women. Healthy cells express ~20,000 Her2+ receptors/cell, however; cancer cells have >100 X the expression of these receptors/cell leading to uncontrolled growth of cells. Trastuzumab is the only monoclonal antibody which specifically binds to the extracellular domain of the Her2+ receptor. It induces G1 cell cycle arrest, reduces proliferation and induces apoptosis. However, many cancer patients develop Trastuzumab resistance to Her2+ breast cancer cells. In this study, Se was attached to the Trastuzumab (~20 µgSe/mg protein) and compared to native Trastuzumab and selenite to assess the Her2+ breast cancer cell line BT-474 viability. Se was attached and measured on the protein by ICP-MS.

Methods: BT-474 cells were cultured using standard protocols and incubated with 4.8 -19.6 µgSe/mg protein concentrations of Se-Trastuzumab, Trastuzumab alone and selenite for 72, 96, 120 & 144 hours. Trypan Blue (TB) (10ul/well) was added to the cells and photographs were taken using an EVOS microscope. An MTT assay was also performed to assess cell viability.

Results: Compared to the control cell growth, Se-Trastuzumab, Trastuzumab and selenite, increased the rate and % of cell TB staining. Instead of G1 cell cycle arrest, Se-Trastuzumab photographically appears to rapidly disrupt BT-474 cell membranes. The MTT assay indicated decreases in cell viability by Se-Trastuzumab as compared to Trastuzumab and selenite treatments.

Conclusions: Se-Trastuzumab demonstrated more intense membrane TB staining and decreased cell viability as compared to control, Trastuzumab or selenite treated cells at equivalent times. Se-Trastuzumab may be a more clinically effective treatment for Her2+ breast cancer before or after patients encounter Trastuzumab resistance.

INVESTIGATION OF SepSecS-DEFICIENT BRAIN SUGGESTS INCREASED OXIDATIVE DAMAGE AS A PATHOGENIC MECHANISM

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We performed whole-exome sequencing to identify the genetic defect in Finnish children with suspicion of mitochondrial encephalopathy. Unexpectedly, compound heterozygous mutations were found in the SEPSECS gene, encoding the O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase (SepSecS), in affected children from three families. The patients had microcephaly at birth, severe spasticity, seizures, cerebellar hypoplasia, elevated blood and/or CSF lactate, axonal neuropathy, signs of liver involvement, edema of hands, feet and face and dysmorphic features. We had access to the autopsy brain sample of one of the patients and to gain understanding of the pathogenesis we used Selected-Reaction Monitoring Mass Spectrometry and Western blotting to quantify selected proteins including selenoproteins in the affected brain. The results were consistent with our neuropathological findings of gliosis and loss of myelin and neurons, and suggested that although the selenoprotein levels were only moderately reduced by the SepSecS deficiency, the resulting lower antioxidant defense may have led to the observed mitochondrial involvement in the disease phenotype. Accordingly, we observed markedly elevated protein oxidation in the patient brain sample, indicative of increased oxidative damage.

THE SELENOPROTEIN GLUTATHIONE PEROXIDASE 4 (GPx4) IS REDUCED IN PARKINSON´S DISEASE

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Mitochondrial impairment and oxidative stress are implicated in the pathogenesis of Parkinson's disease (PD). Selenoproteins play a critical role in the antioxidant defense of the brain. However, the selenocysteine residue at the catalytic active site of selenoproteins is highly susceptible to oxidation and covalent modification by dopamine quinone, resulting in inactivation of protein function. Loss of key mitochondrial selenoprotein function is predicted to increase the vulnerability of dopaminergic neurons to degeneration through the accumulation of oxidative damage in neurons. In this study we examined the status of the mitochondrial selenoprotein, GPX4, in models of PD and in the postmortem brains of individuals who died with the disease. We found that in isolated mitochondria oxidized dopamine (dopamine quinone) dose-dependently decreased GPX4 levels, and exposure of intact PC12 cells to dopamine similarly decreased GPX4 immunoreactivity. In the rotenone rat model of PD, we found a marked loss of GPX4 in substantia nigra (SN) before there was a loss of tyrosine hydroxylase immunoreactivity in neurons. This loss appeared to be selective for SN, since GPX4 levels were unchanged in striatum and cortex. In the brains of individuals who died with PD, there was a similar loss of GPX4 in surviving melanized SN neurons, but not in neurons of the oculomotor nucleus in the same sections. These results suggest that in PD (and models thereof) this mitochondrial antioxidant selenoprotein is lost early and selectively in dopamine neurons, possibly due to attack by dopamine oxidation products. (Supported by NIH grant NS059806)

HMG-CoA REDUCTASE INHIBITORS AS MODULATORS OF SELENOPROTEIN EXPRESSION AND REDOX RESILIENCE

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HMG-CoA reductase inhibitors like atorvastatin or cerivastatin are potent suppressors of the mevalonic acid pathway, and thus of cholesterol biosynthesis. The latter effect has led to the widespread use of statins in the prevention of atherosclerosis and coronary heart disease. Concomitantly, statins can elicit severe side-effects especially in muscle and liver, whose molecular origin has remained unsettled. We have investigated the hypothesis that a downregulation of selenoprotein expression may be involved in these side-effects, as the functional maturation of selenocysteine-tRNA has been reported to depend on isopentenylation, which would in turn make it to rely on the mevalonic acid pathway. We have found that statins at nanomolar con-

centrations bear the potential to suppress selenoprotein synthesis in both muscle cells and hepatocytes, with characteristic differences between the two cell types. In H9c2(2-1) myoblasts and in differentiated C2C12 muscle cells, statin administration led to an increased susceptibility of the cells towards hydrogen peroxide and tert-butyl hydroperoxide toxicity. Selenite supplementation had a pronounced effect on cellular resilience against tert-butyl hydroperoxide, but not against hydrogen peroxide, as would have been expected for an outcome dependent on glutathione peroxidase expression. Still, selenite supplementation was unable to annihilate the difference between statin-treated and untreated cells, consistent with a non-competitive mechanism of action. However, mevalonic acid supplementation fully closed the survival gap between statin-treated and untreated cells. Our data indicate that a posttranscriptional regulatory effect on selenoprotein expression may indeed underlie the major side-effect, i.e. myotoxicity, of the most widely prescribed drug class in industrialized countries. However, the prevention of statin myotoxicity with selenium supplements may nevertheless be unfeasible for mechanistic reasons.

METHYL-SELENIUM COMPOUND TARGETING THE ANDROGEN RECEPTOR SIGNALING AXIS FOR TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

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Relapse with castration-resistant prostate cancer after androgen deprivation therapy constitutes a major cause of prostate cancer mortality. The next-generation anti-androgen MDV3100 prolongs overall survival of patients with metastatic castration-resistant prostate cancer. However, patient responses are variable, and survival benefit remains relatively small. Developing effective modality to improve MDV3100 efficacy is urgently needed. Recent evidence suggests that androgen receptor splice variants (AR-Vs) drive resistance to MDV3100. In the present study, we show that methyl-selenium compound downregulates the expression and activity of both the full-length AR (AR-FL) and AR-Vs. The downregulation is independent of androgen and could be attributable to repressed transcription of the AR gene. Co-treatment with methyl-selenium compound and MDV3100 suppresses AR signaling more dramatically than either agent alone, and synergistically inhibits the growth castration-resistant prostate cancer cells *in vitro*. The combinatorial efficacy is observed in not only AR-V-expressing cells but also cells expressing predominantly AR-FL, likely owing to the ability of the two drugs to block the AR signaling cascade at distinct steps. Ectopic expression of AR-FL or AR-V7 attenuates the combinatorial efficacy, indicating that downregulating AR-FL and AR-V7 is importantly involved in mediating the combinatorial efficacy. Significantly, methyl-selenium compound also downregulates AR-FL and AR-Vs *in vivo* and substantially improves the antitumor efficacy of MDV3100. These findings support a potential combination therapy for improving MDV3100 efficacy, and provide a rationale for evaluating the clinical application of combining methyl-selenium compound with MDV3100 for the treatment of castration-resistant prostate cancer.

EFFECTS OF SELENOMETHIONINE AND SODIUM SELENITE SUPPLEMENTATION ON THE RISKS OF TYPE-2 DIABETES IN Kuo Kondo Alel-y (KKAy) MICE UNDER DIFFERENT STATUS OF SELENIUM LEVEL

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Introduction: Selenium was thought to be beneficial for cancer prevention but recently it has been reported to increase the risks of type-2 diabetes. Selenium status of subjects before supplementation (baseline status) and types of selenocompound supplemented are presumed to contribute to different outcomes. This study aimed to clarify the effects of baseline status and selenocompound types in selenium supplementation to the risks of type-2 diabetes.

Methods: Six-weeks-old KKAy mice were fed high fat diet and classified into deficient (no selenium) and sufficient (selenomethionine 0.1 ppm) groups. From age 8- until 12-weeks-old, mice in deficient group were divided into deficient control (n=10) and deficient+selenomethionine 0.5ppm (n=10) groups; whereas sufficient mice were divided into sufficient control (n=8), sufficient+selenomethionine 0.5ppm (n=10), and sufficient+selenite 0.5ppm

(n=10) groups. Selenium levels, GPx activity, blood glucose, oral glucose tolerance test (OGTT), HOMA-IR, insulin, and adiponectin levels were measured and analyzed by using t test and ANOVA.

Results and discussion: Selenium levels of sufficient group at baseline (8-weeks-old) were higher compared to deficient group [t=1.432 p=0.202 (plasma), t=6.616 p= 0.003 (kidney)]. After supplementation, selenium levels and GPx activities of all supplemented groups increased. Non-fasting blood glucose levels showed significant difference (p=0.044) only at 9-weeks-old. Blood glucose of sufficient+selenomethionine group of OGTT at minute 120 (161.60 ± 76.80 mg/dL) was lower than sufficient control and sufficient+selenite groups, with mean 336.75 ± 57.90 mg/dL (p=0.017) and 306.20 ± 92.90 mg/dL (p=0.035), respectively. Deficient control and sufficient+selenomethionine groups had the lowest HOMA-IR. Adiponectin levels in all groups dropped at the end of study period. Different from supplementation trials in human, these results showed that Se sufficient groups gained benefit from selenomethionine.

TrxR1 and TrxR2 GENE EXPRESSION IN AN ARSENIC TRIOXIDE TREATED APL PATIENT (A CASE STUDY)

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In 2000, arsenic trioxide (Trisenox®, ATO, As2O3) was approved by the US Food and Drug Administration (FDA) for the treatment of patients with refractory acute promielocytic leukaemia (APL), a blood-related malignancy of the white blood cells. Thyoredoxine reductase (TrxR) is a likely cellular target molecule for arsenicals, but it is still unknown what potential importance the targeting of this selenoenzyme is for treatment outcome in a clinical setting. In the present study TrxR1 and TrxR2 isoforms, together with some other selenoproteins, were followed during arsenic treatment of an APL patient with 'normal' serum selenium levels at the beginning of treatment (112ng/g). The patient was treated with As2O3, iv infusion, 0.15 mg/kg body weight per day for up to 50 treatment days. During therapy blood, plasma and urine samples were collected before infusion. Se, As and arsenic species (AsIII, AsV, MMA, DMA) were measured by hydride generation atomic fluorescence spectrometry and/or by radiochemical neutron activation analysis. Real time qPCR was used to follow the whole blood mRNA transcripts of thyoredoxin reductase (genes TXNRD1, TXNRD2), glutathione peroxidase (genes GPX1, GPX3, GPX4) and selenoprotein P (gene SEPP1). We observed a high methylation rate of arsenic, a serum selenium decrease (a drop to 74 ng/g during the first 15 days) and an urine selenium decrease. In parallel a change in blood selenoprotein transcripts was evident for all investigated selenoproteins including TrxR1 and TrxR2. The results undoubtly confirmed the suppressive effects of arsenic on selenoprotein gene expression during clinical arsenic trioxide treatment.

RELATIONSHIP BETWEEN GENETIC POLYMORPHISM OF GPx4 AND SEMINAL DNA DAMAGE IN HEALTHY MEN

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Phospolipid glutathione prexidase (GPx4) is selenoprotein belonging to the family of glutathione peroxidases, and as suggested in variety study is essential for male fertility. GPx4 is expressed in high levels and protects cells from the effects of reactive oxygen species action. Generation of the ROS and sperm lipid peroxidation may have accompanied DNA damage. Seminal DNA damage may be linked with morphological and functional abnormalities of spermatozoa and many of those abnormalities have adversely affected reproductive outcomes. The aim of this study was to examine relationship between genetic polimorphism of GPx4 *718*C/T (rs713041) and seminal DNA damage. Samples collected from 327 normal or slightly oligozoospermic men attending fertility clinics, aged 22-56 yrs were analyzed. Couples' infertility continued for 1-2 years in 36 % of couples, 2-3 years in 33 % of couples, over 3 years in 31 % of couples. The extent of DNA damage in terms of sperm DFI (DNA fragmentation index) or HDS (high DNA stainability index) correlated with semen quality parameters in the investigated men. In the group examined, the GPx4 allele distribution was as follow: 35.4% of individuals were CC homozygotes, 47.3% were CT heterozygotes and 17.3% were TT homozygotes. A multivariate analysis of the relation-

ship between the extent of chromatin damage and genetic polymorphism of GPx4 revealed that in GPx4 718TT homozygote subjects DFI is significantly higher than in GPx4 718 CC homozygotes ($p< 0.045$). It was not found any association between GPX4 718 C/T and HDS level.

FATIGUE RELATING CHANGES OF SELENIUM, ZINC AND COPPER IN SERUM DURING CONTINUOUS WORK LOADING PROCESS

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Introduction: Physiological fatigue is one of the important issues in occupational health field. It is expected to establish the easy technique for detecting the physiological fatigue and to clarify the biological aspects in the fatigue process. The purpose of this study is to examine the effects of physiological fatigue, in continuous fatigue load and the recovery process, on the trace elements such as selenium(Se), zinc (Zn) and copper(Cu) whether changes with fatigue indexes such as the flicker value detected by new flicker measurement system.

Subjects and methods: The subjects (n=12) received a continuous desk work load for 18 hours and rest time (4 hours) after work. The blood samples were collected every 6 hours and end period. The concentrations of Se, Zn, and Cu in serum in the time course of continuous fatigue load and the recovery process were determined by ICP-MS. Three fatigue indexes, ie, flicker value, subjective symptoms, the Visual Analog Scale (VAS) were also examined.

Results and discussion: Flicker value was decreased continuously during the fatigue load, and then increased after taking rest time. Subjective symptoms and VAS were increased continuously during the fatigue load, and decreased after taking rest time. Se in serum showed tendency to increase in the fatigue process after 18 hours and decreased in the recovery process. Zn in serum was significantly increased in the fatigue load process after 18 hours ($p=0.0016$). Cu in serum showed no change in the fatigue process. Se and Zn were suggested to be changed with fatigue process.

THE MODIFYING EFFECT OF *GPX1* AND *SEPP1* POLYMORPHISMS ON OXIDATIVE STRESS MARKERS IN THE SELENIUM SUPPLEMENTED INDIVIDUALS

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In the light of recent epidemiological data suggesting that long term supplementation with selenium does not prevent from prostate cancer and it may increase the risk of diabetes in the individuals with high selenium status, the use of selenium in terms of cancer prevention is not recommended for the general population. Beneficial effects of selenium are probably limited only to the undernourished populations and they are strictly related to dose and chemical form of this trace element. It is also hypothesized, that biological activity of selenium in the human organism may be modified by genetic polymorphism of selected selenoproteins. This may suggest that depending on genotype, some individuals may benefit from selenium supplementation and some may not. Based on this hypothesis, an intervention study was designed in order to assess the influence of the genetic polymorphism of two selected selenoproteins (selenoprotein P, Sepp1 and cytosolic glutathione peroxidase, GPx1) on the markers of oxidative stress in the individuals supplemented with selenium. The supplemented group included 95 individuals aged 18-60 (43 men and 52 women) who were selected for the study according to *GPX1* and *SEPP1* genotypes. From the statistical point of view, this approach allowed us to obtain sufficiently large number of individuals in the groups with rare genotypes. All individuals received 200 μg of selenium in the form of selenium yeast, for six weeks. During the trial, blood for analysis was collected at four time points. The analysis comprised plasma selenium concentration, activities of glutathione peroxidase and superoxide dismutase, plasma ceruloplasmin concentration, total plasma antioxidant capacity, lipid peroxidation (as TBARS), oxidative DNA damage and the production of reactive oxygen species in peripheral blood granulocytes. This work was supported by The Polish Ministry of Science and Higher Education (grant 1666/B/P01/2011/40) and NIOM Internal Grant IMP 1.13/2012).

COMPREHENSIVE SELENIUM STATUS OF POPULATIONS LIVING IN BANDUNG CITY, INDONESIA: DAILY INTAKE, SHORT- AND LONG-TERM SELENIUM STATUS, AND GPx ACTIVITIES

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Selenium is an essential trace element that its deficiencies are often associated to the development of several diseases, such as cancer and cardiovascular diseases. To prevent selenium deficiency-related diseases, it is important to monitor the average of their daily intake in each region. The information on the daily intake of selenium will benefit the authorities as an early warning to decide if the populations need any selenium fortification program to prevent the selenium deficiency-related diseases. Indonesia is a country with over 250 millions of populations, with a high prevalence of cardiovascular diseases. However in our knowledge, study on the selenium status of Indonesian population is never conducted. Thus, this research aimed at knowing the selenium status of Indonesian population.The study has conducted a measurement of food consumption survey, an analysis of short- and long-term selenium status, and a determination of GPx activities of the populations. We have obtained the first information about selenium status of Indonesian populations, especially those who lives in Bandung City. This information become an important database that can be useful to the treatment of selenium deficiency-related diseases in our country. The result may also become a guideline for a further policy to increase the selenium level of the populations.

EFFECTS OF SELENIUM ALONE OR IN COMBINATION WITH INSULIN OR EXENDIN-4 ON THE EXPRESSION OF INSULIN RECEPTOR SIGNAL TRANSDUCERS AND ON GLUCOSE TRANSPORTERS IN DIABETIC RATS

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The mechanisms by which selenium exerts its effects in diabetes mellitus are still unclear. Here, we investigated the effects of selenium alone or in combination with insulin, or exendin-4 on the expression of glucagon- like -1 receptor (GLP-1R), insulin receptor (IR) signal transducers and glucose transporters in tissues of streptozotocin-induced diabetes in male rats. Seven groups of rats were employed: control rats, diabetic rats, diabetic rats treated with 5 ppm selenium in drinking water as sodium selenite, insulin, exendin-4, a combination of selenium with insulin or a combination of selenium with exendin-4 for 5 days. We determined the expression of GLP-1R, IR, insulin receptor substrate-1 (IRS-1), V-raf-leukemia viral oncogene 1 (Raf-1), protein kinase C (PKC), pyruvate dehydrogenase kinase isozyme 1 (PDK1), phosphatidylinositol 3-kinase (PI3K), glucose transporter-2 (GLUT 2), and glucose transporter-4 (GLUT 4) in the liver and kidneys from all groups of rats in this study. Understanding the effects of selenium alone and in combination with other drugs such as insulin, exendin-4 on the expression of these genes will enable the designing of new treatment strategies for the treatment of diabetes mellitus.

EFFECTS OF A CONGENITAL CYTOMEGALOVIRUS INFECTION ON THE SELENIUM STATUS OF MOTHERS AND NEONATES

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Background: Published data on human enterovirus infection and bacterial sepsis indicate that low selenium levels are associated with increased pathogenicity/virulence and mortality, respectively. Cytomegalovirus (CMV) is considered to be the most common congenital viral infection in the developed

world. Infected infants have a high risk for severe neurologic sequelae. The objective of this study was to determine whether there is an association of CMV-infections with selenium status.

Methods: A retrospective case-control study was conducted. One hundred and one (n=101) mothers and their newborn infants were included. Blood from the mothers and from the umbilical cords after birth were sampled. Seven mothers with their infants were tested positive for CMV (CMV-positive, n=7). Trace element concentrations were determined by total-reflection X-ray fluorescence (TXRF) analysis. Statistical analyses were performed using SPSS version 19. Differences in trace element concentrations between CMV-positive versus CMV-negative mothers as well as between CMV-positive versus CMV-negative infants were compared.

Results: Characteristic gradients of the trace element concentrations between mother blood and umbilical cord samples were observed. There were no significant differences between the groups of CMV-positive and CMV-negative mothers and infants with respect to total copper, zinc or iron levels.

Similarly, there were no significantly differences of selenium concentrations between the groups of CMV-positive and CMV-negative mothers ($P = 0{,}979$). However, selenium concentrations were significantly reduced in the cord blood of CMV-positive infants as compared to CMV-negative infants ($P = 0{,}007$).

Conclusion: The selenium concentrations of neonates with congenital CMV infection are significantly reduced.

This deficit may impact negatively on child development.Supported by the German Research Council (DFG) and Federal Ministry of Economics and Technology (BMWi).

AEROBIC BIOGENESIS OF SELENIUM NANOPARTICLES BY *BACILLUS MYCOIDES* SeITE01 ISOLATED FROM THE RHIZOSPHERE OF A Se-HYPERACCUMULATOR PLANT

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Nowadays it is well known that several microbial strains are capable of reducing, through enzymatic or non enzymatic mechanisms, the oxidative selenium forms into the elemental one leading to the formation of well-defined nanoscale particles, Se-nanoparticles (SeNPs).SeNPs, due to their biological and physic-chemical properties find interesting applications in the field of nanotechnology. Moreover, they have recently found application as antimicrobial agents against human pathogens. Se-reducing bacteria are ubiquitous and occur in diverse terrestrial and acquatic environments. However, few microorganisms have been well characterized for their ability to reduce toxic Se oxyanions into non toxic elemental form under aerobic and anaerobic conditions. The majority of studies on the biogenesis of SeNPs have focused on anaerobic mechanisms. On the other hand, little is known on the biological synthesis of SeNPs by aerobic bacterial strains. In the present work, a bacterial strain previously isolated from the rhizosphere of *Astragalus bisulcatus*, a Se hyperaccumulator plant, grown on a Se polluted soil was identified as *Bacillus mycoides* through phylogenetic and physiological analyses. SeITE01 strain showed high efficiency in selenite removal from aqueous growth medium - 2.0 mM of selenite removed within 24 h of incubation - and in Se^0 formation as well. In fact nearly 90% of the total selenite initially supplied to the growth medium was transformed into elemental selenium. Moreover, Se nanoparticles formation was studied through TEM-EDX, SEM-EDX and AFM analyses. Spherical SeNPs were detected mainly in the extracellular space, with dimensions ranging from 90 to 250 nm (diameter). Futhermore, enzymatic assays carried out on the different cellular protein fractions showed high selenite reducing activity for the membrane protein fraction. Thus, a mechanism of selenite transformation and SeNPS production by *Bacillus mycoides* SeITE01 strain was tentatively proposed.

SELENOPROTEIN P AND THYROID FUNCTION STATUS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Introduction: Chronic Kidney Disease (CKD) has great impact on thyroid hormone (TH)metabolism resulting in low T3 concentrations in patients with a low glomerular filtration rate (GFR). CKD might affect both pathways involved in renal TH metabolism, i.e. T3 generation from T4 and degradation of reverse T3, which are catalyzed by deiodinase selenoenzymes. Selenoprotein P (SePP) contributes the majority of serum selenium. Data on selenoprotein status in CKD and its implication on TH metabolism in renal tubular epithelium are rare. Therefore, we studied serum SePP and TH status in various CKD stages (1-5 not yet on dialysis) and in chronic hemodialysis patients (CHD).

Methods: 180 CKD patients (stage1-5) and 72 CHD patients on hemodialysis 3 times/week were prospectively investigated for clinical data, parameters of renal function, serum thyroid profile (TSH, T4, fT4, T3, fT3, rT3, TBG), CRP and serum SePP.

Results: In the CKD patients SePP was negatively correlated with T4 ($r = -0.18$; $p < 0.02$) and fT4 ($r = -0.17$; $p < 0.03$) and positively correlated with TSH ($r = 0.17$; $p < 0.03$). Renal function had no impact on SePP levels. SePP was negatively correlated with CRP ($r = -0.17$; $p < 0.03$). As expected, T4 ($r = 0.18$; $p < 0.02$) and fT4 ($r = 0.23$; $p < 0.005$) were positively correlated with renal function. There was no association of SePP with T3, fT3, rT3 or TBG. SePP conc. were significantly lower in the CHD group (mean ± SD): 2.7 ± 0.8 mg/l vs. 3.3 ± 0.9 ($p < 0.001$) in CKD patients; the same observation applies to T4, fT4, T3 and fT3. There was no association of SePP with any of the TH parameters in the CHD group.

Summary and conclusion: Patients with impaired renal function show still normal SePP conc. in the presence of normal but decreasing T4 and fT4 values. In patients on hemodialysis, however, SePP conc. are significantly reduced.

Thus, selenoprotein status seems to have a different impact on TH levels in patients on dialysis compared to patients with moderate renal insufficiency.

LOW Se AND ELEVATED HAIR Mn, Cd: A SPECIFIC FEATURE FOR AUTISTIC CHILDREN?

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The investigation of trace element impact in the pathogenesis of autism and mental retardation remains very actual. We analyzed by ICP-MS the scalp hair samples taken in 29 autistic children (Autistic Spectrum Disorders, ASD), 88 mentally retarded (MR) without ASD. The obtained data was compared to children without neuropsychiatric disorders (n = 220). All children (ca 50% of both sexes) were 5-10 years old. A significant decrease of higher Cu and elevated Pb in both ASD and MR groups was found. MR children also demonstrated significantly low hair Zn, Ca, Mg, P as compared to controls; and a tendency to lower Zn, Cd, Cu and significantly lower Ca as compared to the ASD group. Only for ASD children the elevated hair Mn, Cd and lower Se were typical. So the accumulation of toxic metals such as Mn and Cd can be due to Se deficiency and the derangement of the detoxication mechanisms in autistic children respectively. The individual correction of the evaluated deviations in trace elements metabolism improved the clinical data in children with ASD and MR. Obtained data can explain the clinical effectiveness of detoxication, chelation therapy in some autistic patients.

EFFICIENCY OF A NEW ORGANIC SELENIUM SOURCE (2-HYDROXY-4-METHYLSELENOBUTANOIC ACID or HMSeBA) IN BROILER CHICKENS

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The experiments conducted aimed to study the efficiency of 2-hydroxy-4-methylselenobutanoic acid (HMSeBA or SO), a new selenium (Se) additive and its bioconversion in the animal in comparison with sodium selenite (SS) and Se-yeast (SY) in broiler chickens. The relative bioavailability of the different Se sources was measured through muscle (*pectoralis major*) total Se, selenomethionine (SeMet) and selenocysteine (SeCys) concentrations, and Apparent Digestibility of total Se. In a first experiment, from d0 to d21, Se sources were tested at different dietary levels and compared to unsupplemented diet (NC). The different Se sources and levels improved muscle Se concentration compared to NC; with additional muscle Se concentrations for the different sources as follow: SO>SY>SS ($P < 0.05$). Moreover, a slope ratio model revealed an increased muscle Se concentration of 1.40 (95% confidence interval, 1.28 - 1.49) for SO compared to SY, equivalent to a bioavailability improvement by 40%. Seleno-amino acid speciation results for NC, SY and SO at 0.3 mg Se/kg feed indicated that muscle Se was only present as SeMet or SeCys, showing a full conversion of Se by the animal and representing a proof of concept for the bioconversion of HMSeBA in broiler chickens.

The second experiment (d0 to d24), compared SS, SY or SO at 0.3 mg Se/kg feed. The Apparent Digestibility of total Se measurements carried out between d20 and d23 were 24, 46 and 49% for SS, SY and SO, respectively, with significant differences between the organic and mineral Se sources ($P < 0.05$).

These results confirmed the higher bioavailability of organic Se sources compared to mineral source and demonstrated a significantly better efficiency of HMSeBA compared to SY for muscle Se enrichment.

DISTRIBUTION AND MATERNAL TRANSFER OF MERCURY IN JAPANESE QUAILS INGESTING SODIUM SELENITE

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Selenium (Se) is well known to play a role in detoxification of toxic metals, in particular, mercury (Hg). Although it has been reported that Se detoxifies inorganic Hg by direct interaction in mammals, certain mechanisms of Se for the Hg detoxification in other animals are limited. Several avian species are important for human health because they are bred as a poultry for the consumption of their meat and eggs. Birds belong to higher trophic levels in the biosphere, thus, they may play a significant role in Se and Hg circulation in the ecosystem. In this study, we administrated inorganic Hg ($HgCl_2$) to the female Japanese quails drinking water *ad libitum* containing sodium selenite at a concentration of 5 µg/mL, and evaluated the body distribution and transfer to eggs of Hg. The egg-laying quails were divided into 4 groups; control, Se alone, Hg alone and Se+Hg groups. The quails were administered orally with mercury chloride at a dose of 10 µg Hg/bird 9 days after beginning the Se ingestion. The laid eggs were collected every 24 h, and then tissues/organs and blood were collected at 7 days after the Hg administration. The Se and Hg concentrations in the samples were determined by an inductivity coupled plasma-mass spectrometer (ICP-MS) and a reducing vapor mercury analyzer, respectively. The concentration of Hg in the egg was markedly increased in quails of Hg alone group. However, the Hg concentration in egg of Se+Hg group was similar to the control group. In addition, the Hg concentration in the liver of maternal quails in the Hg alone group was significantly higher in the Se+Hg group. HPLC-ICP-MS analyses showed that Se and Hg in hepatocytosol of Se+Hg group were co-eluted at a protein fraction. These results suggested that Se alleviated the transfer of Hg from the mother to the eggs in quails.

SELENIUM-ENRICHED PROBIOTICS IMPROVES ANTIOXIDANT STATUS, IMMUNE FUNCTION AND SELENOPROTEIN GENE EXPRESSION OF PIGLETS RAISED IN A HIGH-TEMPERATURE ENVIRONMENT

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The objective was to study the effects of selenium-enriched probiotics (SP) on antioxidant status, immune function and selenoprotein gene mRNA levels of piglets under high-temperature environments. Crossbred ([Landrace × Yorkshire] × Duroc) weaning piglets (n=48) were allocated to 4 groups and received an ad libitum 4 dietary treatments respectively for 42 days: (1) basal diet alone, (2) basal diet supplemented with a level of probiotics (P) equal to that of the SP group, (3) basal diet supplemented with 0.3 mg/kg of selenium as sodium selenite (SS), (4) basal diet supplemented with 0.3 mg/kg of selenium as SP. Three piglets were randomly selected from each group for blood samples collection at Day 0, 14, 28 and 42 and for liver, kidney and spleen samples collection at Day 42. These samples were analyzed for antioxidant parameters, T-cell receptor (TCR)-induced T lymphocyte proliferation, IL-2 concentration and GPx1, GPx4 and TR1 mRNA expressions. The results showed that SS and SP could significantly increase GPX activity and mRNA expressions of GPx1 and TR1 ($P < 0.05$). However, GPx4 mRNA expressions were not affected by SS and SP ($P > 0.05$). P, SS and SP could significantly increase SOD activity, GSH content, TCR-induced T lymphocyte proliferation, IL-2 concentration ($P < 0.05$) and decrease MDA content ($P < 0.05$), respectively. SP has the maximum effects of these parameters. In conclusion, SP serves as a potential dietary supplement for piglets under high-temperature environments.

DIETARY SELENIUM AND NUTRITIONAL PLANE ALTERS MATERNAL ENDOCRINE PROFILES DURING PREGNANCY AND LACTATION

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The objectives were to examine effects of Se supply and nutritional plane during pregnancy on placental size at term and maternal endocrine profiles throughout gestation and early lactation. Rambouillet ewe lambs (n = 84) were allocated to treatments that included Se supply of adequate Se (ASe, 11.5 µg/kg BW) or high Se (HSe, 77 µg/kg BW) initiated at breeding and nutritional plane of 60% (RES), 100% (CON), or 140% (EXC) of requirements beginning on d 40 of gestation. At parturition lambs were removed from their dams, and ewes were transitioned to a common diet that met requirements of lactation. From a subset of ewes (n = 42) blood samples were taken during gestation, parturition, and lactation to determine hormone concentrations. During gestation maternal progesterone, estradiol-17β, and growth hormone were increased in RES and decreased in EXC compared with CON ewes. In contrast during gestation maternal cortisol, insulin like growth factor-I, prolactin, triiodothyronine, and thyroxine were decreased in RES and increased in EXC compared with CON ewes. Placental efficiency tended ($P < 0.1$) to be reduced in HSe compared with ASe ewes. Cotyledon number was reduced ($P = 0.03$) in RES and EXC compared with CON ewes. Placental delivery time tended ($P = 0.07$) to be shorter in HSe compared with ASe ewes, whereas placental delivery time was longer ($P = 0.02$) in RES compared with CON and EXC ewes. Se supply did not alter maternal hormone profiles during gestation. During parturition and lactation, maternal hormone concentrations followed similar profiles compared to gestation, even though ewes were transitioned to a common diet. Therefore, nutritional plane during gestation may partially impact lactation performance through altered endocrine profiles.

F ISH OIL AND SELENIUM-VITAMIN E SUPPLEMENTATION OF EGYPTIAN SHEEP AND ITS FINNISH LANDRACE CROSS-BRED DURING LATE PREGNANCY: 1. EWE RESPONSE HAMADA

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The influence of supplemental fish oil and selenium-vitamin E during late pregnancy on the production and immune efficiency of Egyptian sheep and its Finnish Landrace crossbred was evaluated in this study. Fifty-one gestating ewes [native breeds (Ossimi and Rahmani, n = 30) and its crosses with exotic Finnish Landrace breed (n = 21)] were assigned to 3 equal experimental groups of 17 animals each according to breed, age and live body weight. One group was fed a fish oil supplemented diet for 6 weeks prior to lambing (Sardine oil; 40gm/ewe/day); another group was received two injections of selenium-vitamin E (5ml of 2mg sodium selinate and 100mg vitamin E) once 3 weeks and again 1 week before lambing, and a third group was kept as a control. The data recorded include gestation length, live body weight, body condition score and maternal behaviour over the first 30 min after birth. Blood samples were collected from each ewe, two week before birth, at birth and two weeks after birth for determination of phagocytic activity and hematological parameters. In addition, colostrum samples were collected at 12h of birth for estimation of constituents. Fish oil and selenium-vitamin E supplementation of gestation ewes resulted in increased gestation length and improved body condition score and acceptance of parturient ewes to lamb sucking attempts. It could be concluded that fish oil and selenium-vitamin E supplementation of gestation ewes resulted in increased gestation length and improved body condition score, acceptance of parturient ewes to lamb sucking attempts and phagocytic activity. Moreover, slightly positive impact was imposed on hematological, immune and metabolic status of supplemented ewes. Conversely, these changes were more particularly evident in native breeds than crossbred.

HUMAN METABOLISM AND RENAL ELIMINATION OF ORALLY ADMINISTERED SODIUM SELENATE

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The recommended daily allowance for selenium (Se) is generally taken up by food in form of organic compounds. Besides, occupational exposure to predominant inorganic salts exist at some industrial workplaces. For the estimation of potential health risks it is important to understand whether and how far the metabolism and elimination for these species are different. The aim of this study was to investigate the toxicokinetics of orally administered sodium selenate in humans. For a defined Se exposure a commercial dietary supplement containing 50 µg Se as sodium selenate was used. The supplement was orally administered to 7 subjects and afterwards their urine was successively collected for 24h. In each sample the total Se content as well as the concentrations for selected Se species were determined. Another 24-h urine sample was collected by each participant without supplementation. The amount of total Se eliminated within 24h after ingestion ranged between 27.3-56.9 µg. The subjects showed a fast renal elimination of Se with a maximum about 2h after intake. Taking into account the individual 24-h background amounts the selenate administration resulted in an additional excretion of total Se of 15.4±3.3 µg. The speciation analysis revealed that with 15.1±3.5 µg Se/24h unmetabolized selenate played a crucial role in the Se elimination after ingestion of sodium selenate. One subject showed an increased elimination of methyl-2-acetamido-2-deoxy-1-seleno-β-D-galactopyranoside with a maximum 5h after supplementation.

Three subjects showed an elimination of trimethylselenium ion (TMSe) in the range of 2.5-13.1 µg Se, whereas in the other subjects this metabolite was not detectable.Generally orally administered selenate is excreted very fast via urine mainly unmetabolized. Individuals who feature TMSe generation transfer selenate to TMSe partly. Only in special cases selenate may be metabolized to Se sugars, which cover the main urinary Se species of the background excretion.

RESPONSE TO METHYLMERCURY OF EUROPEAN SEABASS (*Dicentrarchus labrax*) PRE-EXPOSED TO SODIUM SELENITE - NON-DESTRUCTIVE METHODS TO RECOGNIZE CONTAMINATED FISH AND FISH WELFARE

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Mercury bioaccumulates in the food chain and some fish species have been targeted as a high-risk component of the diet. However, both the risk analyses and the recommendations to adjust the intake of seafood should take into account the ratio Se:Hg of the product, and not only the Hg concentration as it is being done at present. For example, it has been shown that the simultaneous exposition to sodium selenite (NaSe) and MeHg decreases or eliminates the negative effects of MeHg.The purposes of this work are: 1) to identify non-invasive, non-destructive methods to categorize contaminated fish (exposed to 4µg MeHg /l for 2 weeks) and 2) to document the effect of the pre-exposition to NaSe (10µg/l for 1 week) on fish exposed to MeHg (4µg/l for 2 weeks).Our results indicate that *D. labrax* (about 5g, kindly provided by Tinamenor, Spain) exposed to MeHg could be differentiated from control fish by analyzing the evolution of the trajectory of the treated fish-group by linear and non-linear system dynamic features, indicating the promising value of these parameters as indicators of contaminations and of fish welfare. Western blot analysis revealed some differences in the anti-myosin heavy chain reacting material between the control and the groups treated with MeHg. Autometallography with silver enhancement showed, as expected, no signal in controls and a higher signal in samples treated with NaSe and MeHg than in samples treated only with MeHg. These results are in accordance with the potential MeHg-detoxifying role of Se.In summary, analysis of the fish group´s trajectory seems to be suitable to distinguish contaminated from clean fish *in vivo* and without the need to disturb the fish. Pre-treatment with NaSe apparently increased the Hg signal, we assume that through some mechanisms that would induce the production of metallic Hg from the MeHg linked to proteins in the tissues.

INTEGRATED MASS SPECTROMETRIC DETERMINATION OF SELENIUM IN DIFFERENT TISSUES OF RAINBOW TROUT FISH (ONCORHYNCHUS MYKISS) FED WITH SELENIUM ENRICHED DIET

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Bioavailability and toxicity of selenium are dependent on its content and chemical species in which it is present in the dietary source [1]. Selenium is integrated in to several Se-containing proteins (selenoproteins) in form of selenocysteine, many of which are fundamental in cell homeostasis [2]. Selenium is also incorporated non-specifically in to proteins (proteins containing selenium) by substituting sulfur of methionine [1]. Fish is potentially an important source of dietary selenium for humans. Thus this study focuses on selenium distribution in the organs of rainbow trout fed on a selenium-spiked diet. 200 rainbow trout fish of about 70 g weights were divided in to four groups; control, diet A, diet B and diet C.The fish were fed with diets containing selenium concentration ranging from 0.1 -8.5 mg Kg^{-1} for fourteen weeks; four weeks of acclimatization and ten weeks of treatment. Fish were sampled every two weeks of treatment for analysis. The total selenium concentration in different tissues and blood of Rainbow trout were determined using ICP-MS (reaction cell mode) mode by monitoring ^{77} Se and ^{78} Se isotopes by external calibration. Germanium was used as an internal standard. In order to evaluate the accuracy of the method total selenium content in dog fish muscle (DORM - 2) was measured and percentage recovery determined. The highest selenium concentrations were found in kidney and liver. Water soluble protein fraction and selenoamino acids in different tissues were investigated using an integrated technique HPLC-ICP-MS-ESI-MS.

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QUANTITATIVE HPLC/MS STUDY OF SMALL SELENIUM SPECIES IN HUMAN SERUM AND URINE AFTER THE INGESTION OF DIFFERENT SELENIUM SUPPLEMENTS

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To elucidate human selenium metabolism, different body fluids are analyzed for their total selenium content and for their selenium species. Selenium supplements are widely used to increase selenium body status, and many commercial products are available containing different selenium species. We performed seven separate experiments with selenium supplements containing selenate, selenite, selenomethionine, methylselenocysteine or selenized yeast. Each supplement was ingested by one volunteer, except for selenate and selenite where two volunteers were used. Samples of serum (in the first 5 hours following ingestion) and urine (up to 24 hours following ingestion) were collected and analyzed with ICPMS, HPLC/ICPMS, and HPLC/ESI-MS in order to compare the time course of the selenium metabolites from the various selenium sources in these two body fluids.The serum selenium levels increased after ingestion of the supplements containing selenate, selenomethionine or selenized yeast but did not increase when selenite was ingested. Time patterns for total selenium excreted in urine showed rapid excretion when selenate was ingested; selenomethionine and methylselenocysteine were excreted more slowly whereas following selenite or selenized yeast ingestion total selenium values stayed close to the background levels. Additionally, methylselenocysteine and trimethylselenonium ion were identified for the first time in human background serum. When selenate was ingested, selenium was mostly excreted unchanged, whereas for the other supplements selenium was excreted mainly as selenosugar 1.

DETERMINATION OF SOME SELENIUM SPECIES IN SERUM HUMAN BLOOD BY HPLC-ICP-MS

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Nowadays it is well know that into the biological system some selenium species has an important role. Due to there are required analytical method able to differential its species at low levels. The present work shows a method to determinate selenoure, seleno-DL-cysteine, seleno-DL-methionine, seleno-DL-cysteine and Se(metyl)selenocystein human blood. Serum obtained from 10 mL of blood was filtered by 0.5 mm nylon membrane and 50 uL were injected into the instrument. A C_{18} column (symmetry 3.5 mm, 4.6 x 100 um from Water) with a mixture of HCO_2H/MeOH/H_2O (0.1:1.0:98.9) at 1.3 mL/min were used as separation condition. In just five minutes the four selenium species were separated. Three samples shown signal at Se-DL-cysteine time, concentration levels were into 15 to 55 ug L^{-1}. On the other hand, 2 samples from all studied showed an unknown signal near to seleno-DL-cysteine time.

Keyword: Selenium species, blood samples, biological samples.

DISTRIBUTION OF SELENONEINE IN ANIMAL TISSUES AND SEAFOOD

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Selenoneine has been identified to be the major Se compound in fish blood and muscles. Fish might be an important source of Se in the fish-eating population. In order to determine the distribution of Se in animal tissues, levels of selenoneine and overall organic Se were measured in the tissues of fishes and the blood of dolphins and humans by speciation analysis. The method involves monitoring ^{82}Se levels by HPLC-ICP-MS using a GPC column. Selenoneine levels were found to be highest in swordfish muscle (0.22 µg Se/g wet tissue). The selenoneine contents of bigeye tuna, Pacific bluefin tuna, albacore, yellowfin tuna, and alfonsino muscle were 0.10-0.20 µg Se/g. In muscle of these fishes, most organic Se (9-42%) was present as selenoneine. In other fish species, such as Pacific sardine, greeneye, skipjack, Pacific mackerel, horse mackerel, red sea bream, and Japanese barracuda, selenoneine levels were 0.01-0.11 µg Se/g. In addition, canned mackerel products also contained selenoneine at 0.20-0.39 µg Se/g. The mean (min-max) of selenoneine levels in the RBC of striped dolphin, Risso´s dolphin and pantropical spotted dolphin was 3.64 (0.63-6.89) µg Se/g, and found to be the highest level in mammals, and its content to the total Se (4.02 µg /g) was also high (82-96%).To evaluate the potential risks and benefits of fish consumption for health, we also made speciation analysis of Se and Hg for the blood of a fish-eating human population. Concentrations of selenoneine were closely correlated with concentrations of MeHg in the RBCs. The RBC contained 0.510 µg/g total Se, 0.21 µg Se/g selenoneine, and 0.26 µg Se/g Se-containing proteins, whereas the serum contained 0.174 µg/g total Se. Selenoneine is the major chemical form of Se in the RBCs of this fish-eating human population and might be an important biomarker for Se redox status.

DETERMINATION OF TOTAL SELENIUM AND SOME SELENIUM SPECIES IN HUMAN SERUM SAMPLES FROM DIABETIC FOOT PATIENT BY ICP

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It is well known that Se is an essential trace element of important to human biology and health, due to it play an elementary role into immune system and enzyme one; they often require vitamins and minerals such as Zn, Mg, Cu and Se. Nowadays, measuring Se levels in biological fluid is very common and necessary, selenium species even more so. Seleno-DL-cysteine is one of organic selenium species related to human blood samples. For this reason the present work it is shown a selenium determination in human serum samples from foot diabetic patient and control people. Serum obtained from 10 mL of blood was filtered by 0.5 mm nylon membranes and 50 mL were injected into HPLC-ICP-MS system and replicates of each sample were carried out by ICP-OES instrument. Values from the total analysis and the species one were correlated between them.

Keyword: Selenium, selenium species, human serum, diabetic foot.

METHYLMERCURY DETOXIFICATION BY THE SELENONEINE-MEDIATED EXOSOMAL SECRETORY PATHWAY

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Dietary intake of selenium was suggested to reduce methylmercury (MeHg) toxicity in the 1970s. Here, we report the molecular mechanisms of MeHg detoxification by the novel selenium-containing imidazole compound, 2-selenyl-trimethyl-histidine, selenoneine. (Yamashita and Yamashita, *JBC*, 285, 18134-18138, 2010). This compound has a strong radical scavenging activity, and is incorporated by a specific transporter, organic cations/carnitine transporter-1 (OCTN1). We characterized that both selenoneine and OCTN1 mediated MeHg detoxification by the exosomal secretory pathway in zebrafish embryos. The embryos at 8 h post fertilization were microinjected with MeHg-Cys (0.2 ng Hg/embryo) into yolk sac, and cultured in embryonic water for 24 h. The secreted exosomes released into rearing water from the embryos were collected by ultracentrifugation at 100,000 x g for 3 h, and their mercury and selenium contents and protein components were examined. The exosomes released in rearing water contained exosomal marker CD63, endosome marker rab5, lysosomal and autophagic proteins (MAP1-LC3B, cathepsin L, exosomal serine protease), molecular chaperones (HSC70, CDC48), OCTN1 transporter, and ceramide, and the exosomal formation was enhanced by MeHg exposure, and such cellular function was accelerated in the presence of selenoneine. In addition, the treatment of embryos with H^+-ATPase inhibitor, bafilomycin A1, and the knockdown of ATG7, CDC48 or OCTN1 genes enhanced MeHg toxicity and caused severe apoptosis in central nervous system in the MeHg-injected embryos. Therefore, the exosomal secretory pathway triggered by endosomal sorting complexes required for transport (ESCRT) might involve in MeHg detoxification.

2,4-DIHYDROSELENOQUINAZOLINE DERIVATIVE (3a) INDUCES APOPTOSIS AND INHIBITS AUTOPHAGY IN MCF-7 CELLS THOUGH THE Akt/mTOR PATHWAY

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In our previous work, we reported new pyrido[2,3-*d*]pyrimidine and quinazoline derivatives as antitumoral agents[1]. In an effort to guess how these derivatives work, a seleno-quinazoline was chosen for further studies. This work reveals that 2,4-dihydroselenoquinazoline derivative (3a) impinged on the PI3K/Akt/mTOR/S6 ribosomal protein signaling pathway by suppressing the phosphorylation of S6 ribosomal protein in MCF-7 cells. This was associated with decreased cell viability and cell cycle arrest in G_2/M phase. 3a also induced cleavage of both PARP and caspase-7 alongside accumulation of LC3-II and SQSTM1/p62 in MCF7 cells, suggesting that 3a induces apoptosis and inhibits autophagy. Since induction of autophagy has previously been described as a mechanism by which some breast cancer cells counteract proapoptotic signalling and develop resistance to hormone therapy[2], this suggests that the development of potent seleno-quinazoline derivatives may be beneficial in preventing and overcoming chemoresistance in breast cancer. The data also show the complexity of the mTOR signaling pathways which are far from being understood.

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NOVEL THIO- AND SELENO-UREA DERIVATIVES WITH POTENT IN VITRO ACTIVITIES AGAINST SEVERAL CANCER CELL LINES

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Our recent reports have demonstrated potent antitumor activity for several classes of organo-Se compounds, particularly where Se atom is present in functional groups such as selenomethyl, selenocyanate, isoselenocyanate, diselenide, selenol or selenourea. We have now designed a series of seleno- and corresponding thio-urea derivatives of several biologically active heterocyclic scaffolds such as acridine, anthracene-9,10-dione, benzo[*d*][1,3]dioxole, 5-nitrofuran, 2*H*-chromen-2-one and quinaxoline. The *in vitro* effects on cell viability was assessed using a colorimetric MTT assay at 24, 48 and 72 h in several cancer cell lines, including pancreatic (Panc-1 and BxPC-3), melanoma (1205Lu) and colon (HCT116) cancer cell lines. Most of these derivatives reduced cell viability in a dose dependent manner; derivatives of 2*H*-chromen-2-one and anthracene-9,10-dione moieties were more effective, with EC_{50} values ranging from 5 to 25 μM in all of the cell lines tested at 72 h. Interestingly, while in most cases Se compounds were more effective than the corresponding sulfur analogs, in the case of anthracene-9,10-dione scaffold, thiourea analog (VA8G) was more cytotoxic, particularly in pancreatic cancer cell lines tested (EC_{50} values of 6.6 and 7.6 μM versus 43.3 and 14.2 μM for the sulfur and Se analogs in BxPC3 and Panc-1 cell lines, respectively, at 72 h treatment). Notably, this analog was very effective in BxPC-3 cells, which are resistant to current chemotherapy compounds and to other derivatives prepared here. Based on these studies we identified two selenourea derivatives (VA7G and VA7I) and a thiourea derivative (VA8G) as the most potent compounds. These agents effectively induced PARP cleavage. Detailed results of these investigations will be presented.

SELENIUM DETERMINATION IN SOME SPECIES OF RODOFICEAS MACROBENTICS FROM CABO SAN ROMÁN, FALCÓN STATE-VENEZUELA

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Selenium is a minerals, that play an important role for the development of tissues and formation of hormones, enzymes, among others important molecules, it has been reported in some plants that are used in daily diet. Algae are food sources due to its content of proteins, lipids and minerals. In this study, levels of selenium were measured in 11 macrobenthic species rodofíceas from Cabo San Román, Falcon State, Venezuela. The dried seaweed samples were treated and Se concentration was measured using ICP-OES. In species *Bryoptamnion triquetrum* and *Hidropuntia cornea* were not detected, while *Hypnea sp*. one showed higher values 40.56 mg/Kg, this value is higher than the one obtained from spinach (9.82 mg/kg), which is the richest vegetable in mineral. Se concentration levels in the evaluated algae are below to the maximum allowable by Food International Organizations, it suggests that these Algae are a potential food product.

Keywords: Selenium, Rhodophyta, Macrobentic algae, Cabo San Roman, ICP-OES
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SELENITE EXACERBATES INSULIN RESISTANCE IN MOUSE MODELS OF TYPE 2 DIABETES AND OBESITY THROUGH OXIDATIVE STRESS-ACTIVATED JNK PATHWAY

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While selenium (Se) has been shown as an insulin-mimetic, recent studies suggest a positive correlation between high Se intake or plasma Se levels and diabetes. In this study, the effects of sodium selenite treatment on insulin sensitivity and signaling were investigated in mouse models of type 2 diabetes and obesity. In the mice fed a high-fat diet (HFD) and treated with streptozotocin, a nongenetic mouse model of type 2 diabetes, selenite (2mg /kg body weight) administered for 4 weeks impaired the insulin tolerance, and elevat-

ed the fasting plasma insulin levels. Moreover, selenite treatment resulted in decreased glycogen levels and impaired IRS-1/PI3-K/Akt signaling in liver. Selenite treatment also led to increased phosphorylation of ASK1, MKK4 and JNK, in parallel with elevated ROS levels in liver. Furthermore, the HFD-fed obese mice gavaged with selenite exhibit increased fasting plasma insulin level, impaired insulin tolerance, and hepatic insulin signaling. Mechanistically, selenite treatment caused elevated ROS levels, and ASK1/MKK4/JNK signaling in the liver of obese mice. Taken together, the results suggest that sodium selenite treatment exacerbates insulin resistance in mouse models of type 2 diabetes and obesity, which could be mediated, at least in part, by oxidative stress-activated JNK signaling pathway in the liver tissue.

THE EFFECTS OF SELENIUM ON THE IMMUNE SYSTEM OF SALMONIDS: A COMBINED BIOMOLECULAR AND CHEMICAL APPROACH TO INVESTIGATE THE IMPACT OF THIS TRACE ELEMENT ON FISH WELFARE

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Selenium (Se) is an essential trace element, required for efficient function of numerous proteins, including some involved in immunity. Its augmentation may effectively improve animal immune function, and represents an alternative strategy for disease control in aquaculture. The aim of this study is to determine the possible impact of a Se-yeast based additive (Sel-Plex®) on the fish immune response, trying to delineate the bio-molecular mechanisms behind the interactions of this trace element with the fish immune system.We have characterized ten genes encoding for selenoproteins belonging to the glutathione peroxidase (GPxs), thioredoxin reductase (TrxRs) and selenoprotein P (SelPs) families in salmonids. The responsiveness of GPxs, TrxRs and SelPs to Se exposure has been studied in vitro using a trout liver cell line (RTL), to validate their possible use as biomarkers for Se intake in vivo. Afterwards, a ten weeks feeding trial was carried out with rainbow trout given diets containing different concentrations of Sel-Plex®. Every two weeks tissues were collected to measure the expression of selenoproteins and immune-related genes by qPCR. In parallel, ICP-MS was used to quantify the ratio of Se intake/absorption, and HPLC to determine the main Se-metabolites produced in trout from Sel-Plex® intake. All the biomarkers selected were responsive to Se in vitro. TrxRs and SelPs were also induced in vivo, and their transcript expression was inversely correlated with the concentration of Sel-Plex®. These two selenoproteins were selected for antibody production, to further investigate the response at the protein level. TrxRs transcript level was also correlated with the expression of pro-inflammatory genes. Liver and kidney were confirmed to be the most relevant organs in Se metabolism, and several different Se-metabolites accumulated mainly in the hepatic tissue. However, muscle had the highest Se retention, even at low Se level in the diet.

EFFECT OF SOME FACTORS OF VARIATION IN THE CONTENT OF SELENIUM IN THE CAMEL MEAT

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The dromedary is able to produce meat and milk in arid conditions and its production is appreciated by the autochthon population.This study focuses on the determination of selenium content in meat samples obtained at the slaughterhouse of Ouargla (South East Algeria) from 41 males and 20 females camels from Tergui, Sahraoui or Naili breeds, aged between 8 months and 13 years old.The average content of selenium was 21,6 µg/100g of wet tissue, what is higher than values currently reported in ruminants .The level of Se was not influenced by age and sex but Tergui breed showed higher values than the two other breeds. No interaction effect was found between the parameters. This breed effect could result from difference in feed composition, but the hypothesis should be confirmed by further research.In conclusion, the meat from camelids in Algeria is a good source of selenium, confirming its nutritional interest for human.

Keywords: camel, meat, selenium, breed.

COMPARISON OF THE TOXICITIES OF SELENIDE AND SELENOMETHIONINE IN YEAST

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Although it is common to use the generic term selenium when speaking of the beneficial or adverse effects of selenized species, it has become apparent that the biological activity of this essential metalloid is largely determined by the chemical form under which it is administrated. To compare the toxicity of organic versus inorganic selenium compounds we used the yeast *S. cerevisiae* as a model cell.In recent years, hydrogen selenide (H_2Se) has emerged as a key intermediate in inorganic selenium metabolism. To decipher the causes of hydrogen selenide toxicity, we screened a library of yeast deletion mutants and showed that among the genes whose deletion caused hypersensitivity to H_2Se, homologous recombination and DNA damage checkpoints genes were over-represented, suggesting that double-strand breaks in DNA were a major cause of selenide toxicity. This genome-wide analysis combined to *in vitro* biophysical and biochemical approaches led to the conclusion that hydrogen selenide induces toxic DNA breaks through a radical-based mechanism which does not involve superoxide ions [1].Selenomethionine (SeMet) is the usual form of organic selenium in animal and human diets. This molecule is considered much less toxic than inorganic selenocompounds. We show however that SeMet is highly toxic when the extracellular concentration of its sulfur analog (Met) is low. Growth inhibition depends on the intracellular SeMet/Met balance rather than on the intracellular concentration of SeMet *per se*. Several strains deficient in DNA repair that were identified as hypersensitive to selenide were assayed for sensitivity to SeMet. They all keep wild-type growth rate properties in the presence of SeMet. On the other hand, a selenide-insensitive Δ*sod1* strain, which is impaired in superoxide scavenging, shows SeMet hypersensitivity. These results suggest that selenide and SeMet metabolisms have different targets. In particular, superoxide ions appear involved in SeMet toxicity without formation of DNA breaks. With selenide, superoxide is not involved while DNA breaks are.

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