POSTER SESSION A

SELENOPROTEIN mRNA CAP HYPERMETHYLATION AND TRANSLATION INITIATION

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Mammalian mRNAs are produced from complex and coordinated biogenesis pathways and acquire 5' terminal 7-methylguanosine (m^7G) cap structures that are essential to every aspect of mRNA metabolism, including localization, translation and decay. Selenoprotein mRNAs contain an in-frame UGA selenocystein (Sec) codon, usually sign of the termination of translation, and thus undergo particular translation recoding. Consequently, these mRNAs have to follow dedicated biogenesis pathways involving conserved assembly proteins and chaperon complexes. We previously established that the assembly mechanism of selenoprotein mRNPs (notably in the 3'UTR) is common with that of small nuclear, and nucleolar ribonucleoparticles (sn- and snoRNPs). Here, we show that selenoprotein mRNAs bear hypermethylated caps and also undergo a similar 5'end maturation pathway to that of sn(o)RNPs. The trimethylguanosine synthase 1 (Tgs1) interacts with selenoprotein mRNAs to hypermethylate their cap and seems to be important for the selenoprotein mRNAs translational recoding. Our findings establish that the mechanism of Tgs1 recruitment is dependent on assembly chaperones also devoted to sn(o)RNA cap modification. We have analyzed the contribution of these factors and of the cap modification on selenoprotein mRNA translation initiation.

P3SECISearch3 AND Seblastian: NEW TOOLS FOR PREDICTION OF SECIS ELEMENTS AND SELENOPROTEINS

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SECIS elements are stem-loop structures acting as the main signal for recoding a UGA codon to selenocysteine. Eukaryotic SECIS elements contain a peculiar kink-turn motif and are located in the 3´ UTR of selenoprotein transcripts. Since UGA is normally a translational stop signal, selenoproteins are misannotated by standard gene prediction programs, and ad-hoc tools have to be developed.We recently built two new computational methods for selenoprotein identification and analysis: SECISearch3 and Seblastian.SECISearch3 replaces its predecessor SECISearch as a tool for prediction of eukaryotic SECIS elements. In this pipeline, the pattern-based predictions of the original SECISearch are accompanied by predictions from new covariance models. These were built by collecting and aligning hundreds of SECISes, and are searched using the programs Infernal and Covels.Seblastian is a new method for selenoprotein gene finding in nucleotide sequences. It utilizes SECISearch3 as the first step, and then tries to identify potential selenoprotein sequences encoded upstream of SECIS elements, by searching for annotated homologues with blastx. Seblastian is able to both identify known selenoproteins and predict new selenoproteins. By applying these tools to diverse eukaryotic genomes, we provide a ranked list of newly predicted selenoproteins together with their annotated cysteine-containing homologues. An analysis of a representative candidate belonging to the AhpC family shows how the use of Sec in this protein evolved in bacterial and eukaryotic lineages.We provide public access to SECISearch3 and Seblastian through webservers available both at http://gladyshevlab.org/ SelenoproteinPredictionServer/ and http://seblastian.crg.es/. This allows for the first time any user (even without any experience in bioinformatics) to perform reliable selenoprotein prediction on custom nucleotide sequences.

SELENOPROTEIN K IS A COENZYME FOR THE ZDHHC6-MEDIATED PALMITOYLATION OF THE IP3 RECEPTOR

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Selenoprotein K (Selk) is an endoplasmic reticulum (ER) resident transmembrane protein that is important for calcium flux during immune cell activation. Whereas the mechanism by which SelK regulated calcium flux was previously unknown, we have identified SelK as a cofactor in the ZDHHC6-mediated palmitoylation of the inositol trisphosphate receptor (IP3R). Our novel data indicate that the IP3R undergoes palmitoylation and that this post-translational modification is required for the stable expression of the IP3R within the ER membrane. Levels of the IP3R are reduced in bone marrow derived macrophages (BMDMs) isolated from Selk$^{-/-}$ mice. Further, culturing BMDMs from wild type mice with 2-bromopalmitic acid, an inhibitor of the palmitoyltransferase family of proteins, not only reduces IP3R palmitoylation, but reduces the total protein levels. Co-immunoprecipitation studies conducted in HEK293 cells indicate that the SH3 binding domain of Selk directly interacts with ZDHHC6, the sole member of the palmitoyltransferase family with an SH3 domain. In Jurkat T cells, stable knockdown of ZDHHC6 leads to impaired T cell receptor mediated calcium flux through the IP3R, but not calcium flux induced with thapsigargin that operates in an IP3R independent manner. Furthermore, stable knockdown of ZDHHC6 in HEK293 cells impairs IP3R dependent calcium flux when caged IP3 is used as a stimulant, demonstrating that the effect of ZDHHC6 deficiency acts directly on the IP3R. These findings indicate that Selk serves as a coenzyme together with the enzyme ZDHHC6 to catalyze the palmitoylation of the IP3R, which requires this modification for stable expression and function.

DISRUPTION OF THE Txnrd1 GENE IN MOUSE HEPATOCYTES CAUSES A SHIFT IN METABOLIC PATHWAYS LEADING TO MORE EFFICIENT DETOXIFICATION OF ACETAMINOPHEN

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Liver is the primary organ for drug metabolism. Currently, Acetaminophen (Paracetamol, APAP) toxicity is the predominant cause of acute liver failure in the USA and Western Europe. Thioredoxin reductase 1 (*Txnrd1)* is an oxidoreductase with antioxidant functions. The enzyme has broad substrate activity, however, its selenocysteine-containing active site can also be easily targeted by electrophilic drugs. Hence, we have recently shown that the APAP-derived cytotoxic metabolite N-acetyl-*p*-benzoquinone (NAPQI), can efficiently inhibit *Txnrd1*, suggesting that not only the glutathione- but also the thioredoxin-system is compromised upon APAP-intoxication (1). Additionally, we showed that *Txnrd1*-deficient livers have a constitutive shift in several metabolic pathways that, in combination, cause a more effective detoxification of APAP. *Txnrd1*-deficient livers have elevated glycogen stores, a limiting factor in the glucuronidation detoxification pathway. The *Txnrd1*-deficient livers also have increased expression of enzymes needed for glucuronidation, reversion of NAPQI back into APAP, glutathione synthesis, glutathionylation and xenobiotic export. Disruption of the *Txnrd1* gene in hepatocytes using a conditional mutagenesis system has previously been shown to cause transcriptional activation of nuclear factor erythroid 2-related factor (Nrf2) regulated genes, partly explaining the metabolic profile change (2). The metabolic shift in *Txnrd1*-deficient hepatocytes is expected to have effects on the systemic bioavailability, toxicity, and redox implications associated with drug challenges. To better understand these systemic interactions, we are profiling proteomic or metabolic shifts in tissues and serum of normal or tissue-specific *Txnrd1*-null mice following drug challenges. Project supported by NIH AG040020 and Blanceflor *Boncompagni-Ludovisi* foundation. 1. Iverson et al. FRBM, June 2013.2. Suvorova et al. PLoS One, July 2009.

SELENOPROTEIN S IS EXPRESSED FROM TWO ALTERNATIVELY SPLICED FUNCTIONAL TRANSCRIPT VARIANTS

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The *SEPS* gene encodes selenoprotein S (SEPS), an ER-resident protein of 189 amino acids with a single proposed transmembrane region. SEPS is carrying the Sec residue at the penultimate position. It plays a major role in the retrotranslocation of misfolded proteins into the cytosol for proteasomal degradation. Functional polymorphisms in *SEPS* have been identified and associated with a number of inflammatory diseases. Two alternative transcript variants of *SEPS* have been detected. Variant 2 is the common and well-studied transcript while variant 1 is only poorly characterized and considered to be non-functional. It is reported to be devoid of a functional SECIS element, thereby unable to support selenoprotein expression (1). Based on our in silico analyses, we hypothesized that both *SEPS* transcript variants encode functional proteins and decided to test their activities in vitro.Both transcripts were found in several human cell lines by qRT-PCR analysis. When applying ER stress to human hepatocarcinoma HepG2 cells, both transcript variants were specifically increased in parallel to increasing SEPS protein levels. Expression vectors for both transcripts were generated and proved functional inducing biosynthesis of recombinant SEPS protein. Reporter genes for SECIS-dependent Sec incorporation were generated with the SECIS elements of both transcript variants. Both SECIS elements were actively catalyzing Sec insertion into the recombinant reporter proteins. We conclude that both transcript variants 1 and 2 encode a functional Sec-containing SEPS protein. The physiological reason for alternative *SEPS* splicing and simultaneous expression of both variants encoding the same SEPS protein remains mysterious and may be related to fine-tune SEPS expression according to Se availability and acute health issues.

References:

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SELENOPROTEIN EXPRESSION IN THE INTESTINAL STEM CELL COMPARTMENT

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Selenium exerts its diverse functions after being incorporated into selenoproteins. However, the exact role of many selenoproteins has still not been elucidated. For some selenoproteins an impact on cell proliferation and differentiation is conceivable. For instance GPx2, TrxR2 and TrxR3 are specifically expressed at the proliferative crypt base of the intestinal epithelium and, thus, co-localize with an active Wnt pathway, which is essential for the continuous renewal of the intestinal epithelium. Intestinal stem cells are prone to DNA damage and, if harmed, most probably develop into cancer stem cells. Therefore, we aimed to investigate whether additional selenoproteins are mainly localized in stem and progenitor cells of the intestinal epithelium and whether a marginal selenium deficiency impairs the stem cell compartment. Epithelial cells were isolated along the crypt-villus axis of the small intestine from mice fed a selenium-adequate or marginally deficient diet. The mRNA expression of selenium-sensitive selenoproteins like Sepw1, Selh, and Gpx1 was significantly down-regulated in selenium-poor cells of both crypt and villus. Most selenoproteins had a higher mRNA expression level at the crypt base compared to the villus, which was even retained under marginal selenium deficiency. The high expression of Sep15 and Selh at the crypt base could be confirmed on the protein level. The basal redox status and the amount of DNA damage of crypt base cells were not affected by the selenium status. However, if selenium-poor crypt base cells were further treated with hydroperoxides they were less capable to cope with this stress. In summary, selenoproteins appear to be important for the intestinal stem cell compartment. The expression of some selenoproteins is retained even under marginal selenium-deficiency, which seems to protect stem cells under basal conditions.

A NON-RADIOACTIVE ASSAY TO DETERMINE THE ENZYMATIC ACTIVITIES OF DEIODINASE ISOZYMES

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The three deiodinases isozymes (DIO1-3) are the most direct connectors between the essential role of selenium within the organism and thyroid hormone (TH) metabolism. These enzymes are responsible for the activation and inactivation of T4 to the active T3 and the inactive rT3, respectively. Furthermore these selenoenzymes control the metabolism of the recently identified novel class of thyronamine hormones and are decisively involved in iodine homeostasis. There is an established and widespread used protocol for measuring deiodinase activities by monitoring the release of radioactive iodide from thyroid hormone I125-tracer molecules as readout. These assays are highly versatile and specific, but rely on relatively costly substrates and radioactive waste disposal and require radiation-specific work procedures. Therefore, we have developed a non-radioactive method based on the historical Sandell-Kolthoff-reaction to determine trace amounts of iodide in solution.All three DIOs were cloned into a selenoprotein expression cassette and subsequently expressed in HEK293T-cells. Specific activities differed between these isoenzymes (approx. 10-400 pmol/mg*min in total homogenates), but were sufficient in all cases for further testing within the non-radioactive setting. Individual deiodinaten pattern of THs revealed the different substrate preferences of these isoenzymes and identified rT3, T4 and T3 as optimal substrates for DIO1, 2 and 3 for further screening approaches. Endogenous DIO1-activity was quantified in murine liver and kidney, as well as homogenates from FRTL5- and HepG2-cells (rat thyrocyte and human hepatoma cell line). The novel method was successfully applied to monitor and establish stable recombinant expression of human DIO1, DIO2 and DIO3 in cell culture and can now be applied to high-throughput screening assays for the identification of isozyme-specific modulators. This study was supported by the Bundesministerium für Bildung und Forschung (No.0315370C).

SELENOCYSTEINE-CONTAINING THIOREDOXIN REDUCTASE 1 IS ESSENTIAL FOR GROWTH OF MOUSE EMBRYONIC FIBROBLASTS IN LOW BUT NOT HIGH CELL DENSITY

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The cytosolic selenoprotein thioredoxin reductase 1 (TrxR1) is believed to play important roles in several cellular pathways and the mouse knockout of the *Txnrd1* gene encoding the enzyme is embryonically lethal. However, no major phenotype was observed in a number of conditional knockout models and the precise essential role for TrxR1 in normal cell physiology has yet to be defined. At times TrxR1 was shown to be dispensable on a cellular level most likely due to its significant redundancy with the glutathione (GSH) dependent enzyme systems. Here we used *Txnrd1* knockout (*Txnrd1*$^{-/-}$) mouse embryonic fibroblasts (MEFs) to study the impact of *Txnrd1* deletion on these cells. When cultured at low density, but not at high density, *Txnrd1*$^{-/-}$ MEFs could not survive in spite of their upregulated GSH-dependent enzyme systems. Under such conditions, massive cell death was observed in these cells, and this could be prevented by the reconstituted expression of selenocysteine (Sec)-containing TrxR1, but not by expression of Sec-devoid Sec-to-Cys substituted variants of the enzyme. We conclude that Sec-containing TrxR1 is required for support of MEFs growth in low cell density, which is an essential cellular role that cannot be fulfilled by the Cys variant of the enzyme. The possible molecular mechanisms for this peculiar cellular phenotype will be discussed.

REGULATION OF SELENOPROTEIN EXPRESSION BY INTERLEUKIN-6

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Selenoproteins play a crucial role in the maintenance of redox homeostasis and are thus important for the microenvironment during development and regeneration. Tissue injury affects the redox homeostasis locally and systemically by an increase in pro-inflammatory cytokine levels. Oxidative stress may both be detrimental to the tissue injury site and essential for the regenerative healing process. In this context, an excessive production of the pro-inflammatory cytokine Interleukin-6 (IL-6) has been described and may shift the balance towards tissue damage thereby hampering regeneration. As selenoproteins are major components of the antioxidative defense system, we hypothesize that IL-6 may directly affect their expression and compromise the selenoprotein biosynthesis machinery. Using transcription factor prediction algorithms we identified putative STAT3 binding sites in several promoter regions including Selenoprotein P (SEPP), Selenoprotein K (SELK), Glutathione peroxidase 3 (GPX3) and type I iodothyronine deiodinase (DIO1). To further validate IL-6 response genes, luciferase reporter gene assays, gene expression profiling and selenoprotein quantification assays were combined. Our results show that promoter activity was severely decreased by IL-6 in DIO1 (~50%), SEPP (~80%), SELK (>40%) and GPX3 (>60%). Focusing on SEPP as the prime Se transporter, its mRNA level was decreased by more than 50% and the SEPP protein expression decreased accordingly suggesting that IL-6 regulates SePP expression directly on the promoter level via STAT3 signaling. These findings indicate a fast and strong effect of IL-6 on selenoprotein expression likely negatively affecting Se organification, Se transport, and finally Se status. Further experiments are aimed to test whether these findings are of clinical relevance.

THE HUMAN SELENOPROTEIN VIMP IS NON-GLOBULAR AND HARBOURS A REDUCTASE FUNCTION IN AN INTRINSICALLY DISORDERED REGION

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Proteins unable to adopt their native structure inside the endoplasmic reticulum (ER) are degraded by the ER-associated degradation (ERAD) pathway to prevent ER stress. During ERAD, the misfolded proteins are recognized by chaperones and subsequently directed to the cytosol for degradation by the 26S proteasome. The human VCP-interacting membrane protein (hVIMP)/Selenoprotein S (SelS) is a selenoprotein of the ER membrane involved in the ERAD pathway. hVIMP has been shown to interact with known components of the ERAD pathway, including the AAA-ATPase p97 that is responsible for "pulling" substrates into the cytosol as they emerge from the retrotranslocation channel. In addition, VIMP has been shown to protect cells against both oxidative and ER stress, and its expression is upregulated during ER stress. Though the link between VIMP and ERAD exists, the exact biological function of VIMP is still not known. In this work, we characterized a cytosolic fragment of VIMP (cVIMP-Cys) biochemically and structurally using analytical gel filtration, CD and NMR spectroscopy in conjugation with bioinformatics. The data reveals a possible reductase function of hVIMP in the cytosol, thereby elucidating a potential new aspect of ERAD and the process of retrotranslocation.

SUPERNUTRITIONAL AND TOXIC SELENIUM REGULATION OF THE SELENOPROTEIN AND NON-SELENOPROTEIN TRANSCRIPTOMES

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In rodents, 5 of 24 selenoprotein mRNAs, such as glutathione peroxidase-1 (Gpx1), are down-regulated dramatically by selenium (Se) deficiency, but the majority of selenoprotein mRNAs are not regulated by Se deficiency. These levels increase sigmoidally with increasing dietary Se and reach defined plateaus at the Se requirement, making them sensitive biomarkers for Se deficiency. These levels, however, do not further increase with super-nutritional Se status, making them ineffective for detection of high Se status. To identify biomarkers for high Se status, we conducted microarray studies with Se-deficient diets supplemented with up to 5 μg Se/g. Rats fed 5 μg Se/g had significantly altered expression of 1193 liver transcripts, whereas

mice or rats fed ≤ 2 µg Se/g had < 10 transcripts significantly altered relative to Se-adequate animals. Four selenoprotein transcripts in the 1193 transcripts were up-regulated by 5 µg Se/g: Selm and Sepw1 up 2.5-fold and Txnrd1 and Gpx3 up 1.5-fold relative to Se-adequate levels. Functional analysis of genes altered by Se toxicity showed enrichment in cell movement/morphogenesis, extracellular matrix, and development/angiogenesis processes. Genes up-regulated by Se deficiency and Se toxicity were targets of the stress response transcription factor, Nrf2. Lastly, individual expression values for 6 transcripts regulated by 2 µg Se/g plus 6 transcripts regulated by Se deficiency were used to develop a molecular biomarker panel for predicting the measured liver Se. This resulted in a panel of 11 significant transcripts, with an overall correlation coefficient of 0.9988 ($P< 10^{-6}$) that accounted for 99% of the variation in liver Se concentration over the full range from 0 to 5 µg Se/g. These studies show that Se toxicity in rats vastly alters the liver transcriptome whereas Se-deficiency or high but non-toxic Se intake elicits relatively few changes, and that these changes can accurately predict super-nutritional and toxic Se status.

REDOX PROTEINS IN THE DEFENCE AGAINST DOPAMINE INDUCED CELL DEATH

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Background: Although the etiology of sporadic Parkinson disease (PD) is unknown, it is well established that oxidative stress plays important roles in the pathophysiology. The thioredoxin and glutaredoxin systems are two central systems upholding the sulfhydryl homeostasis by reducing disulfides and mixed disulfides within the cell and thereby protecting against oxidative stress. In the present study we used the dopamine metabolite 6-hydroxydopamine (6-OHDA) to model PD and to explore the protective effects of these two systems. The powerful neurotoxin 6-OHDA is highly prone to oxidation, resulting in the formation of the 6-OHDA-quinone, a highly reactive species.

Results: In human post-mortem PD brains, the levels of thioredoxin 1 and thioredoxin reductase 1 were found to be significantly decreased. The neuroblastoma cell line SH-SY5Y and the nematode *C. elegans* were used as model systems to evaluate the toxic effects of 6-OHDA. Selenite supplementation protected neuroblastoma cells against 6-OHDA, possibly by upregulating the selenoprotein thioredoxin reductase. A knock-down of thioredoxin and thioredoxin reductase by siRNA resulted in increased cell death in SH-SY5Y. Furthermore, both the thioredoxin and the glutaredoxin systems were able to reduce 6-OHDA-quinone. The reduction required the dithiol mechanism as glutaredoxin with a mutation in the C-terminal cysteine of the active site was found to be ineffective in reducing 6-OHDA-quinone. To further investigate the protective role of proteins belonging to the thioredoxin system, experiments were conducted in *C. elegans* with 6-OHDA treatment of null mutants for *trxr-1*, *trx4* or *trx5* followed by evaluation of their neuronal integrity.

Conclusions: Our results suggest that the glutaredoxin and the thioredoxin systems appear to be important for neuronal survival in dopamine induced cell death.

ROLE OF SELENIUM IN B CELL RECEPTOR FUNCTIONS

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Selenium is known to regulate the functions of B cells such as antibody production and class switching. In addition, selenium can impact the function of B cells by its effect on T cells. However, the role of selenium in B cell receptor (BCR) functions, critical for normal B cell development and secondary immune responses, have not been well established. We investigated the effect of selenium supplementation on BCR endocytosis and fate of antigen-receptor internalization using murine B cell lymphoma expressing human IgM specific for phoshorylcholine. Our data indicate that the rate of endocytosis of BCR was significantly accelerated in the presence of selenium. Furthermore, Anntigen-BCR complex were significantly enhanced in the presence of selenium. Initial signaling events down stream of BCR was also up-regulated in the presence of selenium. Consequently, antigen presentation by B cells was enhanced in the presence of selenium. These data together suggest that selenium, likely via selenoproteins, may regulate the B cell receptor-antigen trafficking and processing to impact the secondary immune responses. Further studies identifying the selenoprotein/s involved in the BCR trafficking and antigen processing and presentations are in progress.

Sep15 INTERACTS WITH RDH11 AND AFFECTS ITS ENZYME ACTIVITY

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15 kDa selenoprotein (Sep15) was firstly described in 1998 in human T cells, and then identified related to various tumors. SEP15 gene knock out mouse showed nuclear cataract, but the mechanism was not clear.The role of Sep15 in brain has not been understood so far. Yeast two-hybridization was used to find out a possible interaction protein of Sep15, retinol dehydrogenase 11 (RDH11) from the human embroy cDNA library. To verify their interaction, fluorescence resonance energy transfer (FRET), Co-Immunoprecipitation (co-IP) and pull down assays were performed. Acceptor bleaching assays (one way of FRET) showed that the protein-protein interaction between Sep15 and RDH11 was verified with 29.21%±6.1% of FRET efficiency and 5.51nm±0.235nm of distance. The results of co-IP and pull down assays also verify the protein-protein interaction.RDH11 is an enzyme depending on NADPH to reduce all-*trans* retinal to all-*trans* retinol. So the yield of retinol in vitro was detected by HPLC, which indirectly reflected the enzyme activity change of RDH11. The results suggested that the activity of RDH11 decreased when Sep15 was over expression. Retinol is involved in visual cycle and retinoid signaling pathway, so Sep15 possibly regulates the reductase activity of RDH11 through the interaction with RDH11 to keep the balance of the amount of retinol and retinal in organisms.

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EFFECTS OF HIGH FAT, SELENIUM-DEFICIENT, AND HIGH-SELENIUM DIETS ON DIABETES BIOMARKERS IN WILDTYPE AND GLUTATHIONE PEROXIDASE-1 NULL MICE

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Supernutritional selenium (Se, 4X the RDA) in humans is associated with risk of diabetes. In mice, Se supplementation above the requirement is reported to elevate diabetes biomarkers (DB), and glutathione peroxidase-1 (Gpx1) overexpression causes obesity and higher DB. We recently reported that high Se in mice increases transcripts associated with increased reactive oxygen (Nrf2) and altered glucose metabolism (BMC Genomics 12:26 (2011)). Thus high fat, such as in US diets, might modulate impact of Se and Gpx1 on DB. Weanling (4.5 wk old) Gpx1 null (-Gpx1) and wildtype (+Gpx1) mice were fed 0, 0.1, 0.8 or 2.0 µg Se/g as selenite with low (11% cal, LF) or high fat (45% cal, HF) for 12 wk. High Se did not increase body weight whereas HF increased weight 11%. Genotype had no effect on weight in LF mice but HF increased weight in +Gpx1 mice fed 0 µg Se/g. Fasting glucose, glucose tolerance and fasting insulin were decreased by Se, and increased by HF; these effects were blunted in -Gpx1 mice, and only increased by HF in +Gpx1 mice. Unexpectedly, DB in Gpx1 heterozygote (+/-Gpx1) mice fed 0 µg Se/g were indistinguishable from -Gpx1 mice. In summary, HF, Se-deficient and high-Se diets had little impact on DB in -Gpx1 mice; HF with high Se had little impact in +Gpx1 mice, but HF in Se-deficient +Gpx1 mice elevated DB but not in ±Gpx1 mice. These results suggest that modulation of peroxide tone by diet or genotype can lead to glucose disregulation. (UW Foundation Selenium Nutrition Research fund)

SELENIUM SUPPLEMENTATION ON DISTRIBUTION OF SELENIUM AND OXIDATIVE STRESS IN BREAST TUMOR-BEARING MICE

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Background: Selenium (Se), an essential micronutrient, has antioxidant activity, anti- inflammatory and anti-carcinogenic properties. D*isruption* of S*e homeostasis* may increase the risk for development and progression of breast cancer. Present work was carried out to investigate the effects of Se administration on distribution of Se and oxidative stress status in breast tumor-bearing mice.

Design and Methods: The EMT6 mouse mammary tumor cells were injected subcutaneously to

each female BALB/cByJNarl mice. At day 8, animals were randomly assigned into TB (0 ng Se/day by gavage), TBLS (22.78 ng Se/day, given as a Se yeast), TBMS (45.57 ng Se/day), and TBHS (91.12 ng Se/day) groups. After 14-day experimental period, tumor growth and Se concentrations and malondialdehyde (MDA) products in plasma, various tissues and tumors were measured.

Results: Compared with the TB group, breast tumor-bearing animals treated with Se have significant increases in plasma Se concentrations and decreases in erythrocyte MDA products. The concentrations of Se in various tissues including hepatic, lung and brain of Se-treated animals were remarkably higher. The concentrations of tumor Se in these Se-treated animals increased significantly compared to the TB group in a dose-dependent manner. Higher MDA products in tumors were observed as tumor Se concentrations increased; whereas the MDA products significantly lower in various tissues than the corresponding values found in the TB animals. Se concentration in tumors was also positively related to the tumor MDA products. Further, higher tumor Se accumulation deceases breast tumor size.

Conclusions: Se accumulation in the tissues may protect against oxidative damage but high tumor Se-induce oxidative stress attenuate the breast tumor burden.

SELENOPROTEIN S PROTECTS VASCULAR SMOOTH MUSCLE CELLS FROM APOPTOSIS INDUCED BY OXIDATIVE STRESS AND ENDOPLASMIC RETICULUM STRESS

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Vascular smooth muscle cells (VSMCs) apoptosis caused by oxidative stress and endoplasmic reticulum (ER) stress (two forms of cell stress) is the important event during the development of atherosclerosis, which will lead to atherosclerotic plaque instability and rupture. Selenoprotein S (SelS) is an ER-resident protein involved in the ER stress response. Previous studies showed that polymorphisms in the SelS is associated with cardiovascular disease, but the underlying mechanism remains unknown [Hum Genet 2007,122:355-365; Acta Diabetol 2013,50:391-399]. In this paper, the influence of SelS on oxidative stress and ER stress-induced VSMCs apoptosis was studied. The result showed that H_2O_2 or tunicamycin, a well known ER stress inducer, induced VSMCs apoptosis. Meanwhile, H_2O_2 or tunicamycin activated ER stress, as demonstrated by the increased protein levels of SelS, ER chaperone GRP78, ER stress transducer phosphorylated PERK, and the proapoptotic transcription factor CHOP. SelS gene silence by siRNA significantly aggravated VSMCs apoptosis and ER stress induced by H_2O_2 or tunicamycin. Moreover, SelS gene silence enhanced H_2O_2-stimulated reactive oxygen species generation. In conclusion, these results suggested that SelS might protect VSMCs from apoptosis by regulating oxidative stress and ER stress, which provided mechanistic insights about the relationship between SelS and cardiovascular disease.

Keywords : Selenoprotein S; vascular smooth muscle cells; apoptosis; oxidative stress; endoplasmic reticulum stress.

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ROLE OF EMT IN MODULATING GROWTH INHIBITORY AND APOPTOTIC RESPONSES IN TUMOR CELLS BY SODIUM SELENITE

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Sodium selenite has *in vitro* proven to be a potent anticancer agent on malignant tumor cells, at doses that have little or no effect on benign cell lines. In addition, sodium selenite appears to have superior cytotoxic effects on drug resistant tumor cells when compared to drug sensitive cells. The toxicity is further dependent on the extracellular thiol status of the cells, which is partly regulated by the xc^- antiporter. Epithelial to mesenchymal transition (EMT) is a latent developmental process that is re-activated in tumor cells and is associated with invasion, metastasis and drug resistance. In this study we aimed to explore if EMT represents a mechanism which sensitizes tumor cells to selenium. The lung cancer cell line A549 that undergoes EMT after exposure to TGF-b1 was used as a model system. Our results show that EMT cells are more sensitive to sodium selenite induced cytotoxicity, and that the sensitization might be a result of an altered thiol status, as the EMT derived cells had a more reduced extracellular environment compared to the control cells. Moreover, our findings demonstrate a down regulation of the cystine regulating xc^- antiporter explained by a decrease in CD44, which stabilizes the subunit on the xc^- antiporter on the plasma membrane. The expression of thiol-redox proteins belonging to the thioredoxin and glutaredoxin systems was altered upon EMT in these cells.

Taken together, our results suggest that selenite is a potential cytotoxic agent against metastatic cancer cells *in vivo*.

A CHEMILUMINESCENT COMPARISON OF THE REDOX CHEMISTRY OF SULFUR, SELENIUM AND OXYGEN ANALOGS

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Purpose: To compare the Lucigenin-dependent Chemiluminescence (CL) for the activity of oxygen (O), sulfur (S) and selenium (Se) containing analogs to generate superoxide (O2-).

Background: Some sulfur and selenium compounds are known to be cytotoxic when present as sulfides; isothiocyanates (RS-, RCNS) and selenides; isoselenocyanates (RSe-, RCNSe). Cytotoxicity of these compounds often correlates with the generation of the superoxide (O2-) anion. We compared different S, Se and O compounds with respect to their activity to generate (O2-) and CL.

Methods: To the chemiluminescence (CL) cocktail was added S, Se or O compounds and CL was integrated over 10 minutes in a Turner Luminometer at 36°C. Stock solutions (2 mg/mL) of the S, Se or OH moiety of the compounds were prepared in methanol. Volumes corresponding to 0.0625, 0.125, 0.250 and 0.500 mg of S, Se or OH moiety of benzyl (BZ) thiocyanate, BZ selenocyanate, BZ isothiocyanate, BZ isoselenocyanate, BZ alcohol, 1-butanol, 1-pentanol, 4-amino-1-butanol and 5-amino-1-pentanol were added to the 500 µL of cocktail. Below shows the mean CL ±SD (N=20) for 0.0625 mgs of each compound respectively. The remaining data to be presented.Blank 0.0590 SD 0.014BZ thiocyanate 0.114 SD 0.052BZ selenocyanate 0.068 SD 0.040BZ isothiocyanate 0.100 SD 0.049BZ isoselenocyanate 0.702 SD 0.323BZ alcohol 0.074 SD 0.040Butanol 0.040 SD 0.031Pentanol 0.053 SD 0.0414-Amino-1-butanol 0.054 SD 0.0435-Amino-1-pentanol 0.156 SD 0.113.

Results: As shown above, BZ isoselenocyanate generated the most (O2-), followed by 5-amino-1-pentanol. At 0.125 and 0.250 mg the BZ isoselenocyanate redox cycled the most followed by BZ thiocyanate. At 0.500 mg, 5-amino-1-pentanol redox cycles the most followed by 4-amino-1-butanol. These amino alcohols that redox cycle may have some potential pharmaceutical applications.

IMPACT OF CADMIUM ON ANTIOXIDANT ENZYMES IN HCT116 CELLS AND PROTECTIVE INTERACTION BY SELENIUM

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Cadmium (Cd) is carcinogenic, but underlying mechanisms are complex and not completely understood. Increased oxidative stress by inhibition of reactive oxygen species (ROS) scavenging antioxidant enzymes is one proposed mechanism. Within this present study, we investigated whether selenium (Se) dependent enzymes (glutathione peroxidase (GPx) and thioredoxin reductase (TrxR)) as well as Se independent enzymes (superoxide dismutase (SOD) and catalase (CAT)) are potential targets of Cd. Human colon carcinoma (HCT116) cells were grown under standard cell culture conditions and were exposed to 0-50 µM $CdCl_2$ for 24h. Cd significantly decreased the GPx activity, while it only marginally inhibited TrxR activity at the highest tested concentration. By supplementing the cell culture medium with 50 nM selenite, the impact of Se on Cd-mediated effects was studied. Basal GPx activity was more than 300 % higher in Se supplemented cells, while basal TrxR activity was not significantly higher under Se supplementation. Furthermore, both GPx and TrxR activities were restored in Se supplemented cells after exposure to Cd. Regarding CAT and SOD enzyme activities upon Cd exposure, only CAT activity was significantly decreased. We further investigated whether Se supplementation could potentially enhance overall cellular antioxidative capacity by modulating CAT and SOD enzyme activities. However, Se supplementation did neither alter their basal enzyme activities nor restore Cd-induced CAT activity inhibition. Long-term cytotoxicity was also investigated by determining colony forming ability, which showed no effect of Se supplementation after exposure to Cd. Taken together, Cd exposure led to an inhibition of ROS scavenging enzymes GPx and CAT in HCT116 cells. Se supplementation restored GPx activity, indicating that selenocysteine in the active center of GPx may be a sensitive target of Cd.

WHEN SULFUR IS MORE CONVENIENT THAN SELENIUM: INSIGHTS FROM GPx7 KINETICS

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Mammalian cells contain two monomeric GPxs (7 and 8) containing a peroxidatic Cys (C_P) in place of Sec (CysGPxs). They lack the resolving Cys (C_R) of nearly all other monomeric CysGPxs, which confers reactivity with thioredoxin (Trx). As the latters, they are structurally related to the monomeric SecGPx4. Are these 1CysGPxs involved in reducing "harmful hydroperoxides" or is the peculiar Cys-containing active site involved in other functions?GPx7 and GPx8 are located within the endplasmic reticulum (ER), where GPx8 is a trans-membrane protein. Both have been reported oxidizing PDI, but not GSH.On chimeric human GPx7 we calculated the rate constants for the oxidative and reductive steps of the peroxidatic cycle. This showed that: a) oxidation rate of C_P is similar to that measured for other 2CysGPxs ($9.5 \times 10^3 M^{-1}s^{-1}$); b) both, PDI and GSH, are reducing substrates, while Trx is not; c) the integrated rate constant of the reductive step, is 3.5×10^3 $M^{-1}s^{-1}$ for PDI and 12.6 $M^{-1}s^{-1}$ for GSH, i.e. the reaction with GSH severely limits the overall cycle. The specificity of the PDI-GPx7 interaction was validated by Surface Plasmon Resonance analysis. Notably, the affinity of GSH is higher for GPx7 than for GPx1 or GPx4, and the specific activity is comparable when PDI or GSH are in the physiological range -i.e. 5µM PDI or 3mM GSH-. On the light of the concentrat on of GSH and PDI in the ER a substrate competition, as a peculiar function, can be proposed. Thus, the slow rate of GSH-mediated reduction of the C_P-SG disulfide emerges as functionally relevant since it allows the regulation PDI oxidation rate by ER GSH concentration. Notably, this is specifically permitted by the use of Cys as the redox residue, since a catalytic Sec would support a much faster reaction with GSH, as occurs indeed in SecGPxs. In conclusion, the presence of catalytic Cys in GPx7 is seemingly functional to allow a specific function that could not be carried out by a catalytic Sec.

ANALYSIS OF SELENOPROTEIN GENE REGULATION IN *METHANOCOCCUS MARIPALUDIS*

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Proteins containing the 21st amino acid, selenocysteine (sec), occur in the three domains of life, Archaea, Bacteria and Eukarya. While the mechanism of sec biosynthesis and incorporation was characterized for representatives of each domain, regulation of selenoprotein gene expression is still poorly understood. The archaeal model organism *Methanococcus maripaludis* contains at least seven selenoproteins known to be involved in its primary metabolism, hydrogenotrophic methanogenesis. Interestingly, the organism also forms cysteine (cys) -containing isoforms of all selenoproteins with the exception of formate dehydrogenase. These isoforms seem to represent a backup system for the organism's ability to conserve energy during selenium starvation. Upon disruption of the selenoprotein biosynthesis pathway, the expression of selenoprotein genes is dramatically downregulated, while exactly the contrary observation for those of the cysteine isoforms can be made. Disrupting *hrsM*, which encodes a LysR-type transcription regulator, results in the same phenotype, suggesting its involvement in regulation of both the sec- and cys-encoding isogenes. Here, we present preliminary efforts to elucidate regulation of selenoprotein gene expression in Archaea in more detail. To this end, *hrsM*-dependent gene regulation was characterized by means of complementation and Western blot analysis allowing the quantification of HrsM encoding transcripts and of the protein in various genetic backgrounds.

EFFECTS OF AMINOGLYCOSIDE ANTIBIOTICS ON SELENOPROTEIN P BIOSYNTHESIS

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Despite their severe side effects, aminoglycoside antibiotics (AG) are used in the treatment of life-threatening bacterial infections. AG provoke misreading during mRNA translation in prokaryotes. To a lesser extent, this disturbance of translation also affects the treated patient. Stop codons are misinterpreted and over-read by insertion of amino acids. This side effect might be of special pathophysiological relevance for the synthesis of selenoproteins, because their essential selenocystein (SeCys) residue is coded by the UGA "stop" codon. The AG-induced misinterpretation likely leads to an inactive selenoenzyme. The resulting selenoprotein deficiency may severely hamper a fast and efficient convalescence of the AG-treated pa-

tient.As the Se status is decisively controlled by Sec-containing selenoprotein P (SePP), we tested the hypothesis that AG treatment reduces Se content of SePP. HepG2 cells were treated with Geniticin and Gentamycin. Characteristic time- and dose-dependent effects on the amount of immunoreactive SePP were observed, reflecting a strong, Se-independent induction of biosynthesis. Specific immunoprecipitation of secreted SePP revealed a dose-dependent decrease in Se content per SePP molecule in response to AG treatment. Biosynthesis of Se-poor SePP upon AG treatment was successfully reduced by increasing the selenite concentration in the culture medium.Our results indicate that AG interfere with regular SePP biosynthesis by catalyzing the incorporation of non-Sec residues at UGA codons. Importantly, these effects can be counteracted for by increasing the Se supply to the hepatocytes in culture. In case the same mechanism is active in AG-treated humans, controlling and correcting their Se status may be an important adjuvant treatment option to reduce side effects. This study was supported by the DFG.

115-kDa SELENOPROTEIN INVOLVED IN ER STRESS OF HepG2 CELLS INDUCED BY tBOOH

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15-kDa selenoprotein was published related to various cancers. ROS (reactive oxygen species) was identified to be higher in tumor tissues than in normal tissues.To study the role of Sep15 in hepatocarcinoma cell lines, HepG2. The cells were treated with or without exogenous oxidative stress, tert-bulty hydroperoxide (tBOOH), then real time PCR and western blot were used to evaluate the changes of Sep15 expression levels. The results showed that Sep15 protein level increased in lower concentration of tBOOH, but decreased in higher concentration of tBOOH. In order to study how Sep15 involved in HepG2 adapting to tBOOH, overexpression and deficiency of Sep15 were employed in HepG2 respectively, and the cell viability was detected by CCK-8 and flow cytometry. Compared to control cells, overexpression of Sep15 in HepG2 increased the early apoptosis of cells, and tBOOH induced more late apoptosis of cell. More apoptotic HepG2 cells were also identified when Sep15 expression in HepG2 cells was knocked down by RNAi and the cells were treated with tBOOH. Sep15 was localized at endoplasmic reticulum and involved in some ER stress. Oxidative stress also induced ER stress. Did changes of Sep15 expression lead to more serious ER stress and induce apoptosis? The ER stress marker Bip and CHOP were detected by western blot.. Overexpression of Sep15 induced Bip expression level and tBOOH treatment furhter increased Bip level. Deficiency of Sep15 increased CHOP protein level. tBOOH treatment increased Bip and CHOP expression levels. In collusion, changes of Sep15 expression level in HepG2 cells induced ER stress. When cells were exposure to oxidative stress, they led to more serious ER stress and increased apoptotic cells.

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LOCALISATION AND DISTRIBUTION OF SELENOPROTEIN P IN HUMAN BRAIN IN HEALTH AND DISEASE

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Aims: The aim of our study was to investigate the presence and distribution of selenoprotein P (SePP) in the human brain. Furthermore we were looking for alterations in neurodegenerative and neuroinflammatory diseases (multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer´s disease, Parkinson´s disease).

Methods: We used a highly sensitive, polymer-based immunohistochemistry system with two different polyclonal rabbit SePP-antibodies. Immunohistochemistry was performed on tissue slides of the BrainNet Europe brain bank.

Results: SePP showed a specific and reproducible distribution in the human brain and localized primarily in different and characteristic intensities to the myelin sheets and neurons. Especially the pyramid neurons of the cortex cerebri and hippocampus and the Purkinje cells of the cerebellar cortex showed strong immunoreactivity. In addition, the long ascending and descending tracts of the spinal cord demonstrated a strong reactivity localized in the myelin sheets and most probably correlating to their degree of myelination. In neurodegenerative and neuroinflammatory diseases subtle differences in these staining patterns were detected.

Conclusions: We have identified the Se-rich SePP to display a highly specific distribution throughout the human brain and spinal cord. Furthermore we were able to demonstrate alterations in some of the studied neuro-

degenerative and neuroinflammatory diseases. These results match very well to recent studies on SePP-knockout mice, which show a complex neurologic phenotype with an atactic gait disorder, and to subsequent studies in Alzheimer´s disease.Further studies will have to reveal whether altered SePP expression or distribution in different brain regions can be correlated to certain neurodegenerative diseases and whether this relationship might offer a promising starting point for new therapies influencing disease progression.

HIGH DIETARY SELENIUM INTAKE ALTERS LIPID METABOLISM AND PROTEIN SYNTHESIS PATHWAYS IN LIVER AND MUSCLE OF WEANLING PIGS

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Our previous research has demonstrated that prolonged high intakes of dietary Se induced gestational diabetes in rats and hyperinsulinemia in pigs. Using pigs as a model of humans, we conducted the present study to explore metabolic and molecular mechanisms for the observed diabetogenic potential of the high dietary Se intakes. A total of 12 weanling pigs were divided into two groups (n = 6/group), and fed a corn-soybean meal basal diet supplemented with Se (as Se enriched yeast) at a normal (0.3 mg/kg) or high (3.0 mg/kg) concentration for 11 wk. Biweekly analyses of plasma glucose and lipid concentrations showed no consistent effect of dietary Se, but pigs fed 3 mg Se/kg had 61% higher ($P < 0.05$) plasma insulin concentration than those fed 0.3 mg Se/kg at wk 11. The high Se diet up-regulated ($P < 0.05$) lipogenesis gene expression (liver: Srebp1 and Fasn; muscle: p53 and Pparg) and hepatic fatty acid synthase activity, but down-regulated ($P < 0.05$) lipolysis gene expression (liver: Cyp7a1; muscle: Acc). Meanwhile, the high Se diet induced ($P < 0.05$) production (P70) or phosphorylation (phosphor-S6) of hepatic proteins involved in the protein synthesis pathway, but exerted no effect on the translation initiation factor elF4E, or mTOR production and phosphorylation. The high Se diet elevated ($P < 0.03$) mRNA levels of Gpx3 in liver and muscle, decreased ($P < 0.05$) mRNA levels of Selw in liver and Sels, Diol1, and Txnrd1 in muscle, and enhanced ($P < 0.05$) GPX activity in liver and muscle as well as GPX3 protein ($P < 0.05$) in liver. In conclusion, feeding pigs with the high Se diet for 11 wk induced mild hyperinsulinemia and up-regulated lipogenesis and protein synthesis pathways. Exploring the relationship between these changes and the altered expression and function of the 6 selenoproteins may help unveil the mechanism for the diabetogenic potential of high Se intake(supported in part by NIH DK 53018).

THE IMPACT OF AGING AND PHOTO-AGING ON DNA REPAIR CAPACITIES IN HUMAN SKIN KERATINOCYTES AND THE PROTECTIVE EFFECT OF SELENIUM

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Few studies have focused on the protective role of selenium (Se) on skin aging and photoaging although selenoproteins are essential for keratinocyte function and skin development. To our knowledge, the impact of Se supplementation on skin cells obtained from elderly and young donors has not been reported. So, the main objective of our work was to evaluate the effects of Se supplementation (as its inorganic form sodium selenite (NaSe)) on skin keratinocytes at baseline or after exposure to UVA irradiation Keratinocytes were obtained from normal skin biopsies of elderly (60-70 years old) or young (20-30 years old) donors and were pre-treated with 30 or 240 nM of NaSe for 72h, followed or not by different doses of UVA. Se concentrations were determined by ICP-MS, cell survival was examined by using both MTT and clonogenic cell survival assay, activities of cytosolic GPx (GPx1) and cytosolic TrxR were determined spectrophotometrically and DNA repair capacities were evaluated by a multiplexed DNA biochip patented and developed at our laboratory We showed that low doses of NaSe ($\leq$ 30 nM) were very potent protector against UVA-induced cytotoxicity on young keratinocytes, whereas the protection efficiency of NaSe on old keratinocytes was obtained only at higher concentrations ($\geq$ 240 nM). Also, we have shown a drastic fall in DNA repair capacities on old keratinocytes versus young one at basal state or after UVA exposure. Supplementation with selenium enhances significantly global DNA repair capacities, especially on those isolated from young donors. It seems that selenium is able to increase OGG1 activity, a glycosylase responsible for the repairing of 8-oxoGua. These original data strongly suggest an increased vulnerability of keratinocytes with age against photoaging and should be taken into account regarding Se needs in elderly. Strengthening of DNA repair activities by selenium may represent a new strategy to fight against aging and skin photoaging.

EFFECTS OF INADEQUATE SELENIUM SUPPLY ON LIVER METABOLISM IN C57BL6/J

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Selenium (Se) is an essential trace element in mammals, which mainly exerts its functions being incorporated into selenoproteins. In Europe, the majority of the population does not reach the Se supply, needed to optimize selenoprotein expression. Thus, suboptimal Se status prevails. To investigate possible effects of inadequate Se supply on liver thyroid hormone status and metabolome, male C57BL6/J mice were fed either a Se adequate (0.15 mg Se/kg) or Se inadequate (0.086 mg Se/kg) diet starting directly after weaning for 7 weeks with the main Se form being selenomethionine. Changes in Se status were confirmed by decreased activity of total liver glutathione peroxidase of mice fed a Se inadequate diet, resulting in elevated levels of 4-hydroxnonenal as a surrogate for oxidative stress. In addition, activity of the selenoprotein Dio1 was reduced to 50% in these liver homogenates, in line with the tendency of a decreased 3,3,'-5-triiodothyronine/thyroxine ratio. The liver metabolome was measured using GC-MS analysis. Amino acids and derivatives were quantified using the iTRAQ Kit. Carbon-one metabolites such as betaine, dimethylglycine (DMG) and choline were analysed by LC-MS/MS. Se poor liver was characterized by significantly elevated levels of hypotaurine, taurine, homocysteine (Hyc) and glutamate, while methionine levels were unaltered. Betaine/choline and DMG/choline ratios tended to be reduced in mice with poor Se status, while betaine/DMG ratios were unaffected. Expression analysis of enzymes involved in Hyc metabolism showed a marked decrease in cystathione-β-lyase (CBS) under Se inadequate conditions. In summary, a low Se status can be linked to higher Hyc levels in the liver which appears to be caused by an impaired transsulfuration pathway indicated by lower CBS levels. Whether the observed oxidative conditions and/or disturbed thyroid hormone status in Se deficiency are crucial for the observed hepatic metabolic changes needs to be further investigated.

TOWARD INTERPRETING THE NULL RESULTS OF SELECT: DIRECT COMPARISON OF THE PROSTATIC TISSUE POTENCY OF SUPRANUTRITIONAL SELENOMETHIONINE VS SELENIUM-YEAST ON MARKERS OF PROSTATIC HOMEOSTASIS AND CANCER RISK REDUCTION

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Supplementation with selenium in the form of selenized yeast (Se-yeast) significantly reduced prostate cancer incidence in the Nutritional Prevention of Cancer Trial. Conversely, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed no such cancer-protective advantage using selenomethionine (SeMet). The possibility that SeMet and Se-yeast are not equipotent in promoting homeostasis and cancer risk reduction in the aging prostate has not been adequately investigated; no direct comparison has ever been reported in man or animals. Here, we analyzed prostatic responses to SeMet or Se-yeast from a controlled feeding trial of 49 elderly beagle dogs-the only non-human species to frequently develop prostate cancer during aging-randomized to one of five groups: control; low-dose SeMet, low-dose Se-yeast (3 μg/kg); high-dose SeMet, high-dose Se-yeast (6 μg/kg). After seven months of supplementation, we found no significant selenium form-dependent differences in toenail or intraprostatic selenium concentration. Next, we determined whether SeMet or Se-yeast acts with different potency on 6 markers of prostatic homeostasis that likely contribute to prostate cancer risk reduction-intraprostatic dihydrotestosterone (DHT), testosterone (T), DHT:T, and epithelial cell DNA damage, proliferation, and apoptosis. By analyzing dogs supplemented with SeMet or Se-yeast that achieved equivalent intraprostatic selenium concentration after supplementation, we showed no significant differences in potency of either selenium form on any of the 6 parameters over three different ranges of target tissue selenium concentration. Our findings, which represent the first direct comparison of SeMet and Se-yeast on readouts in the aging prostate that reflect flux through multiple gene networks, do not further support the notion that the null results of SELECT are attributable to differences in prostatic consequences achievable through daily supplementation with SeMet, rather than Se-yeast.

SELENITE POTENTIATE ALL-TRANS RETINOIC ACID-INDUCED MATURATION OF ACUTE PROMYELOCYTIC LEUKEMIA CELLS

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Acute promyelocytic leukemia (APL) is characterized by t(15;17) chromosomal translocation of retinoic acid receptor α (RARα), resulting in the generation of fusion protein PML/RARα. PML/RARα interferes at the DNA binding sites of PU.1 transcription factor and thereby its functionality. Blockade of the PU.1 mediated transcription leads to clonal proliferation of hematological malignant neoplasm that possesses compromised ability to differentiate into mature leukocytes. Conventionally, either chemotherapeutics or arsenite are used in combination with all-*trans* retinoic acid (ATRA) to treat APL patients. This often results in poor survival associated with side effects. Thus, there exists a persuasive need to develop more effective and less toxic therapies. Selenite is a superior cytotoxic agent to primary leukemic cells *ex vivo* when compared to conventional chemotherapeutics. Also, selenite is a potential inhibitor of zinc-containing transcription factors. In the present study, a similar inhibitory mechanism of selenite on PML/RARα (a zinc-containing protein) is conceived. Together, the present work aims at understanding whether selenite alone or in combination with ATRA can modulate differentiation in a malignant cell line (NB4) of APL origin. Preliminary results suggest that selenite in combination with ATRA potentiates the differentiation in NB4 cells compared to ATRA alone based on the morphological evaluation, myeloperoxidase staining, expression of CD marker and respiratory burst activity. In accordance with our hypothesis, expression of PML/RARα is down-regulated at protein level by selenite alone. However, mRNA expression of PU.1 increases in the combined treatment compared to ATRA alone. We also find out that ATRA modulates the expression of thioredoxin and glutaredoxin family of proteins during the myeloid differentiation. Together, the present investigation suggests the hitherto unknown therapeutic potential of selenite in combination with ATRA in APL.

POTENT INHIBITION OF THIOREDOXIN REDUCTASE AND GLUTATHIONE REDUCTASE BY SILVER IONS *IN VITRO*

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The family of enzymes known as the pyridine nucleotide-disulphide oxidoreductases include both selenium-dependent and independent enzymes in both prokaryotes and eukaryotes. Two of the most well defined enzymes include the mammalian cytosolic thioredoxin reductase (TrxR1) and the glutathione reductase group of enzymes (GR). Interest in the targeting of these enzymes for treatments of several diseases including cancer and certain types of infectious disease has risen in the past ten years. TrxR1 is a selenocysteine containing catalyst that has been shown to be inhibited by various metal and non-metal inhibitors, many of which are important in the clinic today. Our group has recently shown that TrxR1 is potently inhibited by silver ions (Ag) *in vitro*. Human exposure to silver is increasing with the increased use of silver nanoparticles in clothing, wound dressings and surfaces to inhibit microbial growth. Given its inhibition of TrxR1, we next sought to determine whether Ag can inhibit GR as well. Moreover we chose to determine the kinetics of the inhibition of TrxR1 and GR in a comparative study. We found that both enzymes are sensitive to Ag ions as nanomolar concentrations, yet the inhibition of GR by Ag was sensitive to excess thiols (GSH) present in the reaction. In contrast inhibition of TrxR1 by Ag ions was resistant to millimolar levels of GSH, suggesting a very high affinity for the site of inhibition. These data will be presented and placed into context of both toxicology of metals and the potential for insights into drug discovery.

ASSOCIATION BETWEEN PLASMA SELENIUM LEVEL AND Nrf2-REGULATED GENE EXPRESSION IN HUMANS

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Animal studies in rodent and in vitro studies indicate compensatory role of nuclear factor (erythroid-derived 2)-like (Nrf2) and Nrf2-regulated cytoprotective enzymes for the dietary selenium (Se) deficiency or for the loss of selenoproteins. There is no data on Se level and Nrf2 and Nrf2-target gene expression in humans. The observational study was conducted on 103 healthy men aged 18-83 years, to determine the effect of plasma Se level on NRF2, KEAP2 and 16 cytoprotective genes expression in pe-

ripheral blood leukocytes. Transcript levels of *GSTA1, GSTM1, GSTP1, GSTT1 GCLC, GCLM, GSR, NQO1, EPHX1, HMOX1 PRDX1, CAT, SOD1, SOD2, TRXR1* and *GPX2* were determined by means of quantitative Real-Time PCR. Mean plasma Se level was found to be 51.94±16.07 μg/L (range 23.86-96.18 μg/L). All volunteers expressed detectable mRNA levels in blood leukocytes, except from *GPX2* mRNA. The highest level of gene transcripts in circulating leukocytes was observed for *SOD2*, while the lowest was attributable to *KEAP1* gene. Non-smokers presented higher gene expression than current-smokers. *NRF2* mRNA level was positively correlated with expression of investigated genes, except from *KEAP1*, *EPHX1* and *GSTM1*. *KEAP1* mRNA level was positively correlated with *GCLC, GSR, CAT, TRXR1* mRNAs and also with plasma Se level. Inverse relationships between plasma Se level and expression of *NRF2* and Nrf2-regulated genes were found. These significant associations were observed for *GCLM, PRDXR1, SOD1, SOD2, GSTA1, GSTP1, GSTT1* and *TRXR1* genes, only in non-smoking individuals. Results of this study suggest a possible link between plasma Se level and Nrf2-associated cytoprotective response at gene level in humans.This work was supported by NIOM Internal Grant IMP 1.8/2012 and the Ministry of Science and Higher Education (2012/05/B/NZ5/01406).

EFFECTS OF SELENIUM AND EXENDIN-4 ON THE EXPRESSION OF GLP-1R, IRS-1 AND PREPROINSULIN IN THE PANCREAS OF DIABETIC RATS

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The mechanisms by which selenium and exendin-4 exert their antidiabetic actions are still unclear. Here, we investigated the effects of selenium or exendin-4 administration on the expression of glucagon-like peptide-1 receptor (GLP-1R), insulin receptor substrate-1 (IRS-1), and preproinsulin in the pancreas of diabetic rats. Diabetes was induced by streptozotocin administration. Diabetic rats were injected intraperitonially with 0.03 μg exendin-4/kg body weight/ daily or treated with 5 ppm selenium in drinking water for a period of 4 weeks. GLP-1R and IRS-1 levels were decreased while the level of *preproinsulin* mRNA was increased in the pancreas of diabetic untreated rats, as compared to that in control rats. Treatment of diabetic rats with exendin-4 increased protein and mRNA levels of GLP-1R, and IRS-1, and the mRNA level of *preproinsulin* in the pancreas, as compared to their levels in diabetic untreated rats. Selenium treatment of diabetic rats increased the pancreatic mRNA levels of *GLP-1R*, *IRS-1*, and *preproinsulin*. Selenium or exendin-4 treatment of diabetic rats also increased the numbers of pancreatic islets and GLP-1R molecules in the pancreas. Therefore, selenium and exendin-4 may exert their antidiabetic effects by increasing *GLP-1R*, *IRS-1*, and *preproinsulin* expression in the pancreas and by increasing the number of pancreatic islets.

CONCENTRATION OF Se AS AN ESSENTIAL METAL IN HUMAN MILK OF VENEZOLAN WOMEN BY ICP-MS

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Human milk is a biological fluid, which provides all the nutrients, including essential metals that are required for the development and growth of newborn children during the first months of life; also, it contains enzymes, specific proteins, nucleotides, etc. Selenium concentration in milk is directly affected by levels in the food chain, also is a component of two enzymes, glutioneperoxidase thats protects against oxidative damage, and of type I iodothyronine 5-de-iodinase, this antioxidative cellular Se might have a role in preventing Cancer. As essential nutrients, many chemicals can be transferred from body storage sites and the blood of nursing mothers to breast milk. Nowadays, researchs on the importance of essential trace metals, specially Se in human milk, its nutritional quality and its relation with environmental factors are growing. In this work, it is presented the total concentration of Se in samples in human milk using ICP-MS. The samples were collected from breastfeeding women between 2 and 8 postpartum months in the Hospital of Children of Veritas in Maracaibo city. For destruction of organic material, digestion of the real samples with HNO_3 / H_2O_2., both concentrated, was applied. The total metal concentration was 0,011 mg L^{-1} of Se. The limit of detection (LD=3ρ/m) obtained was 0,003 for Se. The precision (expressed as RDS) was < 5%. Accuracy was assessed by recovery studies, obtaining mean recoveries percentages of 100 ± 5%. Developed methods for the determination of total concentration of the studied metal was accuracy, precise and free from interferences.

RUDIMENTS OF WATER

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Experiences over the last thirty years are described in relation to the development of a policy to satisfy the needs of both public water supply and the environment. Examples of the relationship between water resource development and water-dependent environments are described together with the role of demand management. Concerns are raised as to the further expectations of water supply customers, the long- term effect of demand management and the expectations of the regulators and environmentalists.World´s technical and scientific literature on water-related topics covering the characteristics, conservation, control, pollution, treatment, use and management of water resources. technical reports in the physical and life sciences, as well as from engineering, legal and government publications.

THE EFFECT OF LONG-TERM SUPPLEMENTATION WITH SELENIUM-ENRICHED YEAST ON PLASMA LIPIDS

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Background: A recent randomised controlled trials concluded that six months selenium supplementation led to mild decreases in plasma lipid concentrations, when compared to placebo.

Objective: To investigate the effect of long-term selenium supplementation on plasma lipids.

Methods: 491 healthy volunteers aged 60-74, from the region of Funen in Denmark, were enrolled in a placebo-controlled, blinded, parallel-group trial entitled the Denmark PREvention of Cancer by Intervention with SElenium Trial (DK-PRECISE). Participants were randomised to 100 µg (N=124), 200 µg (N=122) or 300 µg (N=119) selenium-enriched yeast or matching yeast-based placebo tablets (N=126) daily for five years. The intervention agent was SelenoPRECISE ® by Pharma Nord A/S. Total- HDL- and LDL-cholesterol, triglycerides and selenium were measured in a non-fasting state, with blood drawn at baseline and after six months and five years intervention.

Results: Baseline characteristics showed no differences between groups. Total cholesterol concentrations decreased significantly in all groups after six months and in placebo, 100 and 200 µg/d groups after five years. HDL decreased significantly in 100 and 300 µg/d groups after six months and increased significantly in the 100 µg/d group after five years. LDL cholesterol concentrations decreased significantly in the 100 µg/d group after six months and in all groups after five years. Neither total cholesterol, HDL, LDL nor triglyceride concentrations differed significantly between any groups after six months or five years intervention (ANOVA or Kruskal-Wallis tests P>0.05). Plasma selenium concentrations increased significantly in treatment groups and differed significantly between groups after six months and five years intervention (ANOVA P< 0.0001) .

Conclusions: Selenium supplementation did not lead to increases in plasma lipid concentrations. Mild decreases in plasma lipid concentrations did not differ between placebo and treatment groups.

[Table 1: Effect of selenium supplementation on pla]

Table 1: Effect of selenium supplementation on plasma lipid and plasma selenium concentrations after six months and five years.

Variable	Placebo	Selenium dose 100 (µg/d)	200 (µg/d)	300 (µg/d)	*P* Value
Total cholesterol (mmol/L)[1]					
Baseline	5.91 (0.87)	6.15 (0.91)	6.01 (0.93)	6.15 (0.91)	
Six months	5.72 (0.80)	5.84 (0.90)	5.87 (0.95)	5.96 (0.86)	0.31[†]
Change from baseline to six months	-0.17 (0.60)	-0.30 (0.55)	-0.14 (0.60)	-0.17 (0.63)	
95% CI	-0.30 to -0.05	-0.41 to -0.19	-0.25 to -0.02	-0.29 to -0.04	
P Value[‡]	0.006	< 0.001	0.02	0.01	
Five years	5.72 (0.84)	5.84 (1.06)	5.66 (0.97)	5.99 (1.19)	0.32[†]
Change from baseline to five years	-0.20	-0.37	-0.45	-0.13	
95% CI	-0.37 to -0.03	-0.53 to -0.20	-0.64 to -0.26	-0.30 to 0.05	
P Value[‡]	0.02	<0.0001	<0.0001	0.07	
HDL cholesterol (mmol/L)[1]					
Baseline	1.63 (0.42)	1.60 (0.33)	1.55 (0.37)	1.61 (0.39)	
Six months	1.61 (0.41)	1.56 (0.30)	1.51 (0.35)	1.55 (0.38)	0.27[†]
Change from baseline to six months	-0.01	-0.04	-0.01	-0.05	
95% CI	-0.05 to 0.02	-0.07 to -0.01	-0.04 to 0.01	-0.08 to -0.02	
P Value[‡]	0.50	0.02	0.34	0.043	
Five years	1.68 (0.45)	1.67 (0.36)	1.57 (0.40)	1.65 (0.42)	0.22[†]
Change from baseline to six months	0.04	0.06	0.00	0.01	
95% CI	-0.01 to 0.09	0.01 to 0.11	-0.04 to 0.05	-0.04 to 0.01	
P Value[‡]	0.15	0.01	0.94	0.77	
LDL cholesterol (mmol/L)[1]					
Baseline	3.63 (0.88)	3.87 (0.86)	3.79 (0.94)	3.88 (0.82)	
Six months	3.53 (0.79)	3.67 (0.84)	3.72 (0.87)	3.84 (0.79)	0.07[†]
Change from baseline to six months	-0.08	-0.19	-0.11	-0.05	
95% CI	-0.19 to 0.03	-0.29 to -0.10	-0.21 to 0.00	-0.15 to 0.06	
P Value[‡]	0.18	<0.0001	0.15	0.47	
Five years	3.48 (0.93)	3.58 (0.91)	3.55 (0.94)	3.74 (1.03)	0.43[†]
Change from baseline to six months	-0.21	-0.38	-0.35	-0.14	
95% CI	-0.36 to -0.06	-0.52 to -0.25	-0.52 to -0.18	-0.30 to -0.03	
P Value[‡]	0.005	<0.0001	0.0005	0.03	
Triglycerides (mmol/L)[2]					
Baseline	1.40 (6.20)	1.39 (4.22)	1.57 (7.22)	1.48 (4.54)	
Six months	1.30 (3.79)	1.43 (3.49)	1.58 (5.04)	1.44 (4.20)	0.15[§]
Five years	1.54 (3.1)	1.43 (2.56)	1.54 (5.34)	1.47 (4.06)	0.57[§]
Selenium (ng/g)[1]					
Baseline	86.3 (15.3)	88.0 (16.5)	88.2 (16.5)	83.5 (17.2)	
Six months	85.5 (14.5)	152.2 (23.8)	208.6 (41.6)	254.1 (55.2)	<0.001[†]
Change from baseline to six months	-1.2	64.5	120.9	170.5	
95% CI	-4.1 to 1.7	59.6 to 69.4	111.9 to 129.9	159.1 to 181.9	
P Value[‡]	0.49	<0.0001	<0.0001	<0.001	
Five years	87.6 (24.6)	157.9 (27.2)	221.8 (41.3)	276.3 (79.3)	<0.001[†]
Change from baseline to six months	0.0	70.7	133.7	191.2	
95% CI	-5.9 to 5.9	63.8 to 77.5	123.9 to 143.6	173.5 to 209.0	
P Value[‡]	0.73	<0.001	<0.001	<0.001	

Legend: Data represent means[1] (SD) and medians[2] (range) in participants with at least one lipid measurement available at baseline, six months or five years selenium supplementation. Two-sided P values are significant at the 5 % level. *P* values are from [†]paired t tests within each group and from [‡]One-way ANOVA F tests for log-transformed parametric continuous variables, and [§]Kruskal-Wallis tests for non-parametric continuous variables, across the four treatment groups. are. 95% CI; 95% Confidence Interval. HDL; high-density lipoprotein. LDL; low-density lipoprotein.

DEVELOPMENT OF HUMAN SELENOPROTEIN P MEASUREMENT SYSTEM AS A POSSIBLE BIOMARKER OF INSULIN RESISTANCE IN TYPE 2 DIABETES

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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. Recently, excess SeP has been suggested to increase insulin resistance and exacerbate type 2 diabetes. SeP may be a therapeutic target of type 2 diabetes and it is significant to evaluate SeP content in this patient. Our research group has been previously developed a sandwich ELISA system for human SeP. In this previous assay system, 2 antibodies, namely BD1 and AH5, recognizing N-terminal fragment were used. The presence of SeP fragments has been reported. Therefore, it is not clear that this previous assay system measure intact SeP and SeP fragments generated from the proteolysis by plasma kallikrein. In the present study using colloidal gold particle solution, measurement system for intact SeP was developed by using AH5 and AA3 antibodies, which recognize N-terminal and C-terminal fragment of SeP, respectively. This assay system was applicable to various clinical analyser. Linearity, precision, and interference of several biological materials was examined. By usng this assay system, it was confirmed that intact SeP levels correlated positively with post-loaded plasma glucose levels in non-diabetic subjects. Circulating levels of intact SeP predicted the future hyperglycemiaindependently of the other clinical parameters. In this presentation, significance of SeP measurement as a possible biomarker of insulin resistance in type 2 diabetes will be discussed.

SELENIDE TARGETS AND PROTECTS INJURED TISSUE

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Because dietary deficiency in selenium results in heart disease and in critically ill patients blood selenium levels are low, selenium is regarded as an essential element required for sustaining tissue health. Using two different mouse models of ischemia reperfusion injury, we found that selenium is concentrated in injured tissues following temporary loss of blood flow while blood selenium levels simultaneously decrease. This targeting of blood selenium to injured tissue is apparent within minutes of restoration of blood flow and is specific for selenide; the oxidized form of selenium, selenite, does not target. Furthermore, intravenous administration of exogenous selenide, but not selenite, after ischemia but before reperfusion significantly protects myocardium in a model of acute myocardial infarction-88% reduction in infarct size. To understand the effect that selenide has on tissues we exposed normal animals to an atmosphere of room air containing 5 ppm of hydrogen selenide gas. We found that oxygen consumption and carbon dioxide production in the animal were decreased by approximately 3-fold and that the effects were reversible when the hydrogen selenide was removed. These results suggest there is a natural mechanism that targets endogenous selenide to recently reperfused tissue and that the protection provided by this targeted selenide may be due to a temporary reduction in metabolism.

Se CONTENT AND EJACULATE PARAMETERS IN INFERTILE MEN

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Se content was determined in ejaculate of 148 infertile and healthy men 19-44 years old by ICP-MS. Semen characteristics (motility, coefficient of mobility, viability, morphology, concentration of spermatozoa, volume) were recoded. It was found that the Se concentration was lower in men with: (1) lower ejaculate volume (< 1.5 ml) - 0.11 (0.05-0.23) vs 0.28 (0.12-0.60) mcg, P< 0.05; (2) lower spermatozoa content (<39x10^6) - 0.17 (0.05-0.41) vs 0.26 (0.09-0.60) mcg, P< 0.1; (3) lower spermatozoa account in 1 ml of ejaculate (< 15x10^6) - 0.19 (0.05-0.042) vs 0.26 (0.09-0.61) mcg, P< 0.04; (4) lower spermatozoa vitality (< 58%) - 0.21 (0.07-0.58) vs 0.21 (0.07-0.67) mcg, P < 0.05; (5) astenozoostermia, oligoastenozoostermia, leucocytostermia vs control (P < 0.05). So, Se determination in ejaculate might be a fruitful diagnostic tool, and Se supplementation can be effective in the treatment of infertile and subfertile men.

THE EFFECT OF SELENIUM DEFICIENCY ON THE LEVELS OF DNA METHYLATION, APOPTOSIS AND INFLAMMATION IN IMMUNE ORGANS OF CHICKS

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Selenium (Se) deficiency influences the normal immune function of chicks, but the molecular mechanism remains unclear. To investigate the effects of Se deficiency on immune function, two groups of day-old layer chicks (n = 60/group) were fed a corn-soy basal diet (33 mg Se/kg; produced in the Se-deficient area of Heilongjiang, China) or the diet supplemented with Se (as sodium selenite) at 0.15 mg/kg for 55d. In the present study, we detected the global DNA methylation level in immune organs (spleen, thymus, and bursa of fabricius) of poultry by using HPLC technology. In addition, the mRNA expression levels of DNA methyltransferase (DNMTs) and methyl binding domain protein 2 (MBD2), expression levels of apoptosis related genes (BCl-2, Capase-3, Bax, P53 and Bak-1), and inflammation related factors (iNOS, COX-2, NF-κB and PTGE) were examined by real-time PCR and Western blot technology. The results showed that the global DNA methylation level, mRNA levels of DNMTs and MBD2, expression levels of apoptosis related genes and inflammation related factors were influenced by Se deficiency. The results showed that Se deficiency may influence the immune function of chicks by affecting DNA methylation, apoptosis or inflammation pathway.

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.

THE EFFECTS OF FRESH WATER AND SEAWATER ADAPTATION ON THE EXPRESSION OF SELENOPROTEINS IN THE MOZAMBIQUE TILAPIA, *OREOCHROMIS MOSSAMBICUS*

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Selenium (Se) is an essential micronutrient for several organisms, including fish. Selenoproteins contain the unique Se-containing aminoacid selenocysteine (Sec), are ubiquitously expressed, and differentially respond to Se levels in a hierarchical, tissue-specific manner. To date, the expression of most selenoproteins has not been verified in euryhaline fish models. The Mozambique tilapia, *Oreochromis mossambicus*, a euryhaline cichlid fish utilized in aquaculture, has high tolerance for changes in salinity and is able to survive in fresh water (FW) and seawater (SW) environments, which differ in Se availability. We searched EST databases for cichlid selenoprotein mRNAs and screened for their differential expression in FW and SW-acclimated tilapia. The expression of mRNAs encoding thirteen selenoproteins was measured in the brain, eye, gill, kidney, liver, pituitary, muscle, and intraperitoneal white adipose tissue (WAT) of male tilapia acclimated to either FW or SW. Expression of mRNAs for the factors involved in selenoprotein synthesis and/or recycling, selenophosphate synthetase 1, Secp43, and Sec lyase, was also measured in the same organs. The highest variation in selenoprotein and synthesis factor mRNA expression between FW- and SW-acclimated fish was found in gill and kidney. Among selenoproteins, deiodinase type 1 and thioredoxin reductase 1 mRNA levels were affected by salinity in most tissues when compared with the other selenoprotein mRNAs examined. Together, our results indicate that selenoprotein expression is differentially affected by acclimation salinity in tilapia and suggest that some selenoproteins may play a role in osmoregulation.

SELENIDE REDUCES OXYGEN DEMAND AND PROTECTS INJURED HEART TISSUE

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Injury following temporary loss of coronary blood flow to the heart is thought to result when demand for oxygen cannot be met by supply. Several human trials show that blood selenium levels fall when oxygen demand exceeds supply following injury to the heart and other tissues. Here we show in mice that inhalation of hydrogen selenide causes a greater than 4-fold decrease in oxygen demand in vivo that is fully reversible when the gas is removed. Furthermore, endogenous selenide is rapidly mobilized from blood to injured tissues following temporary loss of blood flow. And finally, administration of exogenous selenide after temporary loss of coronary blood flow but before reperfusion results in an 88% reduction in infarct size. These results suggest that endogenous selenide is mobilized to tissues after temporary loss of blood flow and that this targeting decreases oxygen demand thereby preventing irreversible damage.

EFFECTS OF SELENITE AND SELENATE TOXICITY AND DELETION OF THIOREDOXIN REDUCTASE IN *CAENORHABDITIS ELEGANS*

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Thioredoxin reductase-1 (TRXR-1) is the sole selenoprotein in *C. elegans*, and selenite is a substrate for thioredoxin reductase, so TRXR-1 may play a role in metabolism of Se to toxic forms. To study the role of TRXR in Se toxicity, we cultured *C. elegans* in axenic media with deletions of *trxr-1*, *trxr-2* and both, with increasing concentrations of inorganic Se. Wild-type *C. elegans* cultured for 12 days in Se-deficient axenic media grow and reproduce equivalent to Se-supplemented media. Supplementation with 0 to 2 mM Se as selenite results in inverse, sigmoidal response curves with an LC_{50} (50% lethal Se concentration) of 0.20 mM Se, due to impaired growth rather than reproduction. Deletion of *trxr-1*, *trxr-2* or both does not modulate growth or Se toxicity in *C. elegans* grown axenically, and ^{75}Se labeling shows that TRXR-1 arises from the *trxr-1* gene and not from bacterial genes. Se response curves for selenide (LC_{50} 0.23 mM Se) were identical to selenite, but sele-

nate was 1/4th as toxic (LC_{50} 0.95 mM Se) as selenite and not modulated by TRXR deletion. These nutritional and genetic studies in axenic media show that Se and TRXR are not essential for *C. elegans*, and that TRXR alone is not essential for metabolism of inorganic Se to toxic species.

MOLECULAR MECHANISMS OF SELENIUM COUNTERACTING ARSENIC TOXICITY

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Millions of people worldwide live in areas where arsenic (As) concentration in drinking water exceeds the WHO guidelines of 0.010 mg/L. Chronic exposure to inorganic As causes arsenicosis, a severe disease characterized by skin and internal cancers and multiple systemic pathologies. There are no proven preventive procedures or treatments for arsenicosis-related cancers. However, it is known from extensive animal studies that selenium (Se) can counteract As toxicity. The molecular mechanism of this effect involves formation of the seleno-bis(S-glutathionyl) arsinium ion in blood, which is rapidly excreted via the hepatobiliary system into the intestinal tract. The multidrug resistance protein MRP2 is critical for transportation of Se-bound arsenic into bile. An inadequate dietary intake of Se and accelerated Se depletion by As are possible contributing factors to arsenicosis. Previous Phase II studies in China and Bangladesh have suggested some clinical benefits of Se supplementation in arsenicosis patients. In conjunction with the recent Phase III clinical trial "Selenium in the Treatment of Arsenic Toxicity and Cancers" (SETAC), we used the SSRL facilities to elucidate mechanisms of As-Se counteractions at the protein, tissue, and organ levels. We employed synchrotron XRF imaging of lyophilized whole-body slices of laboratory hamsters to study distribution of As and Se in different organs after intravenous injection of equimolar concentrations of arsenate and selenite. Chemical speciation of As and Se in freshly frozen tissues of injected animals was determined by XAS. In the next step, we studied the effects of timed exposure to As, Se, and As+Se solutions in MRP2 ± laboratory rats using synchrotron XAS, and observed striking differences in the chemical speciation and excretion rates of As and Se into bile in the wild type and MPR2-lacking animals. These results provide a possible molecular basis for Se palliatives for arsenicosis.

BIOTRANSFORMATION OF SELENIUM IN PLANTS

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Selenium is an essential element for the proper functioning of humans and animals, therefore all researches towards development of dietary supplementation of selenium, which could compensate a natural deficiency of selenium, are considered to be important.It is expected that *Allium cepa*, known as a selenium hyperaccumulator, has the ability to biotransform inorganic selenium compounds into its organic derivatives, namely selenoaminoacids, e.g. Se-methylselenocysteine. Although several investigations were performed with the use of hydroponics cultivation of onions, it was of our interest to compare whether the cultivation in soil will induce similar biotransformation effects. For this purpose the interdisciplinary research involving biological and chemical methods was performed.Thus the aim of this study was to evaluate the biotransformation of different selenium inorganic precursors by onions (*Allium cepa* L.) grown in soil. In particular, identification of selenium organic compounds in plants was of a great interest as this allows understanding of the processes of biotransformation as well as distribution of selenium. High-performance liquid chromatography (HPLC), coupled to ICP-MS was used for this purpose. In order to obtain complementary information, electron microscopy was used. The ultrastructure of roots' tips of *Allium cepa* cells exposed to Se (IV) or Se (VI) was compared. The localization of selenium in plants' cells was examined by using electron microscopy with X-ray microanalyzer. The growth of plants and their biomass as well as mitotic activity was monitored during the cultivation.The developed and validated analytical scenario was used for the determination of selenium and its compounds in plants organs, all towards evaluating the possible mechanism of selenium biotransformation in onions.

VALIDATION OF THE ANALYTICAL PROCEDURE OF DETERMINATION OF SELENIUM IN NEW CANDIDATE REFERENCE MATERIALS

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The aim of this study was to develop and validate method for the determination of total concentration of selenium in the prepared new candidate reference materials. The materials were prepared in the framework of the project "Production and attestation of new types of reference materials crucial for achieving European accreditation for Polish industrial laboratories " - *MODAS* and are responding to the needs of Polish analytical laboratories in the environmental analysis. The most important feature of the produced reference materials will be representativness of the composition of both the matrix and the levels of analytes in comparison to typical environmental samples tested in the Polish analytical laboratories.In this work the method of preparation of samples for testing and the method of determining the total concentration of selenium was presented. At the first stage of this study an optimized microwave digestion procedure was described depending on the matrix components of the reference material. The next stage concerned the choice of the method in order to determine the total concentration of the element as tentative recommended values in new candidate reference material. The determination of the total content of selenium was performed with the use of an inductively coupled plasma mass spectrometry (ICP MS). A verification of the analytical validation parameters of ICP MS such as precision, accuracy, sensitivity, recovery, traceability and uncertainty was done.

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EFFECTS OF PHYSICAL ACTIVITY ON SELENIUM WHOLE BLOOD, SERUM AND HAIR PROFILES IN MEN AND WOMEN

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110 professional sportsmen and 131 sportswomen 17-35 y.o. were investigated (Gr.1). Gr.2 included the same age 157 men and 257 women, regularly visiting fitness centers. 67 men and 157 women with low physical activity were controls (Gr.3). Whole blood (WB), serum (SB) and scalp hair (SH) samples were collected and analyzed by ICP-MS. There were the higher men´s SH Se level in athletes (Me=0,49 ppm) and in Gr.2 (0,48 ppm) vs lower Se level in Gr.3 (0,41 ppm) ($p< 0,1$). There were no significant differences in SH of all three women's groups (0,44 ppm, 0,41 ppm and 0,38 ppm). In WB and SB samples there were no gender differences. Gr.1 had the higher concentrations of Se in SB than people in other groups (0,16 ppm vs 0,14 ppm and 0,12 ppm, respectively). However, there were lower level of Se in WB of athletes than in other groups (0,198 ppm vs 0,204 ppm and 0,209 ppm, respectively). So: 1) The excessive physical activity can cause the Se decrease in WB of men and women respectively. In case of SH and SB Se level can reflects the exertion of immune system. 2) Hair better than WB and SB reflects the long-term effects of physical activity and stress overloading in humans but WB and SB analyses are good tests for short-term effects. The changes in WB and SB Se content are due to the derangement of homeostasis in humans under physical and emotional overloading, typical for athletes. We suppose, that the physical activity can cause the Se redistribution between the blood cells and serum and to derange antioxidant status of the body.

THE EFFECT OF PREPARATION OF PLANT SAMPLES (*ALLIUM CEPA*) ON THE SPECIATION OF SELENIUM

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The organic Se-compounds play an important role in cell biochemistry and in nutrition which is one of the reasons for increased interest in selenium present in food sciences. Therefore a number of research has been done and continues towards investigation of the foods containing Se in an appropriate form, in order to assure the proper benefit for human. Although the knowledge on the content of selenium in food is well documented, there is a lack of information on its chemical forms.Selenium-containing proteins are synthesized by plants and animals. These proteins contain Se-Met and Se-Met-cysteine, the principal form in plants. The Se-content of food is highly dependent on the amount of Se in the soil, which varies geographically and depends on the ability of plants to take up and accumulate the element. In order to en-

rich food with selenium we carried out studies on plants *Allium cepa*, known as a selenium hyperaccumulator with the ability to biotransform inorganic selenium compounds into its organic derivatives, namely selenoaminoacids, e.g. Se-methylselenocysteine. The plants were grown with the addition of sodium selenite in a concentration of 10 mg Se L^{-1} and then the total content of selenium as well as selenium speciation studies were performed.The aim of this study was to investigate the effect of the preparation of plant material (lyophilization or drying) on the existing form of selenium in it. Speciation of selenium in a sample is of great importance due to the use of described plant material as a food product. In order to compare the effect of sample preparation procedure on selenium speciation one part of material was lyophilized and the second part was dried at laboratory conditions at the temperature of 35°C. Total selenium content was determined by ICP-MS after microwave digestion of the sample. The extraction of selenium species was performed with water under different temperature conditions. Speciation of selenium was carried out with HPLC system.

GENE-SPECIFIC REGULATION OF SELENOPROTEINS BY METHYL-IMIDOSELENOCARBAMATES WITH ANTITUMOR ACTIVITY

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Cancer remains a major public health problem and a leading cause of death worldwide. Despite some progress in treatment options, most of the current drugs cause severe side effects. Several studies have indicated that patients with low selenium status have an increased cancer risk. Cancer treatment attempts with selenocompounds have not yet been performed. We have synthesized and studied a series of methyl- and benzyl-imidoselenocarbamates which elicit potent antitumor effects without causing toxicity in mice (1). These effects are potentially mediated by selenoproteins.In order to better characterize their molecular effects, we have compared these selenocompounds in hepatic carcinoma cells with respect to their effects on several selenoenzymes with known redox activities such as GPx1, TxnRd1 and Dio1. In addition, selenoprotein S (SePS) and selenoprotein P (SePP) were analysed as they mediate ER quality control and Se transport, respectively. The methyl-imidoselenocarbamates efficiently stimulated GPx1 and Dio1 activity, SePP secretion and SePS expression. At the same time, TXNRD activity was inhibited. These effects were specific for the methyl-selenocompounds and not observed with the respective benzyl-derivatives. Using HEK293T cells stably expressing DIO1, DIO2 or DIO3, the again only the methyl-selenocompounds increased deiodination activities. These analyses indicate a set of selective and specific effects of the methyl-imidoselenocarbamates on the selenoproteome and support their consideration for clinical studies. In the context of anticancer drug research, especially their efficiency and specificity along with the lack of obvious acute side-effects qualify the methyl-imidoselenocarbamates as promising novel chemotherapeutics which may be considered for further preclinical studies.

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BIOSYNTHESIS AND METABOLISM OF Se-CONTAINING IMIDAZOLE COMPOUND, SELENONEINE, IN ZEBRAFISH EMBRYO

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The novel Se-containing strong antioxidant selenoneine, 2-selenyl-N_α, N_α, N_α- trimethyl-L-histidine, has recently been discovered to be the predominant form of organic Se in tuna blood (Yamashita & Yamashita, JBC, 285, 18134, 2010). A substantial proportion of the total amount of selenium was present as selenoneine in the muscles of ocean fish. Cell growth of human cultured cells were enhanced in the presence of selenoneine at 5-100 nM, and GPx1 gene expression was induced in dose-dependent manner. Therefore, selenoneine is thought to play a key role in the Se redox antioxidant mechanism in animal cells. In order to elucidate metabolic pathways of selenium in animal cells, we examined biosynthesis of selenoneine and selenoproteins in zebrafish embryos. When we zebrafish embryos were cultured in the presence of 0.1 µM [^{76}Se]-selenite Na in Hank´s medium at 28.5C for 3 days, ^{76}Se-labeled selenoneine

was detected in the methanol extract of the embryos by HPLC-ICP-MS analysis. In addition, by metabolic labeling of Se-compounds with [^{75}Se]-selenite, radiolabeled selenoneine was also produced in the zebrafish embryos and purified by HPLC and TLC separation. When we cultured zebrafish embryos in the presence of [^{75}Se]-labeled selenoneine, [^{75}Se]-labeled selenoproteins, such as selenoprotein W and GPx1, were detected by SDS-PAGE. Therefore, selenoneine was found to be biologically synthesized, and incorporated as a selenium source in the zebrafish embryos *in vivo*.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SELENOCARBAMATES AS ANTIPROLIFERATIVE AGENTS

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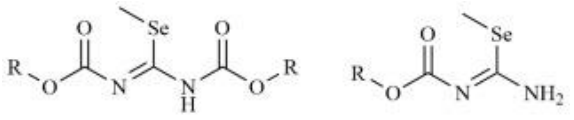
Cancer is a major health problem, being a leading cause of death worldwide. A lack of efficient chemotherapeutic agents is one of the main reasons behind this dismal statistics. Selenium (Se) is an essential trace element that has being widely studied as a chemopreventive agent. Experimental, clinical and epidemiological data confirm that Se and its metabolites, such as methyl selenol, have anticancer effects. Several mechanisms have been suggested to explain the anticancer action: protection against oxidative stress, reduction of DNA damage, cell cycle arrest, angiogenesis inhibition and apoptosis induction being the major ones.[1-3]This study aims the synthesis of a series of new selenocarbamates with structures 1 and the evaluation of their *in vitro* antitumoral activity.

Citotoxicity of the series was tested against a panel of six human cancer cell lines (CCRF-CEM, HTB-54, HT-29, K-562, MCF-7 and PC-3) and two non-malignant cell lines (BEAS-2B and 184B5) by the MTT assay. Tested compounds exhibited antiproliferative activities with GI_{50} values in the micromolar range. Apoptosis induction and cell cycle arrest are also under study. Preliminary data suggest these compounds induce apoptosis in CCRF-CEM cells.

Chemical structures for selenocarbamate:



1

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NOVEL HYBRID DISELENIDES: A NEW CLASS OF POTENT ANTILEISHMANIAL AGENTS

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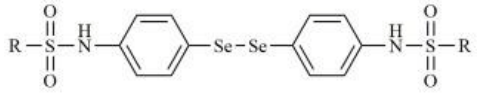
Leishmaniasis constitutes a serious public health problem. According to the World Health Organization, leishmaniasis is now endemic in 88 countries, particularly in subtropical and tropical regions. Selenium is a prominent trace element, whose increased concentration in plasma has been recognized as a new defensive strategy against *Leishmania* infection and after the work developed by our research group[1,2], we realized that diselenide group is important to achieve potential compounds. Besides, sulfonamide compounds present antiparasitic activity including an antileishmanial profile. The design is based on the molecular hybridization between an antileishmania compound, 4,4´-diselanediyldianiline, presented by us in previous paper[2] and sulfonyl chloride compounds. Substituents have been chosen as function of their chemical nature and their activity to modulate bond strength and hydrolysis capacity in the sulfonamide group and also to achieve fragments with potential antiparasitic activity. We carried out the synthesis and biological evaluation of new hybridized diselenides with this general structure:

All the synthesized compounds were subjected to *in vitro* screening against *L. infantum* amastigote model. In order to establish the selectivity index their cytotoxic effect was carried out against THP-1 cell

lines. Compounds, which exhibit the best activity, have been assayed in infected macrophages.

Chemical structures:



R = Heterocyclic and aromatic groups

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DETERMINATION OF SELENIUM LEVELS IN SUBJECTS WITH AUTISTIC SPECTRUM DISORDERS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY: A PRELIMINARY REPORT

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Subjects with autism spectrum disorders (ASD) have a prevalence that seems to be increasing in recent years. An excess or deficiency in natural essentials minerals have been implicated in the etiology of the disorder. The aim of this study was to measure serum levels of essential minerals by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) in 30 serum samples from subjects with ASD and 20 control subjects without apparent neuropsychiatrists conditions. Results of this study showed statistical differences ($p < 0,05$) in Se levels between subjects with ASD and control ones. In conclusion, these results are consistent with other, the decrease of Selenium can be influenced by several factors, among which highlights an imbalance in the homeostasis of this mineral antioxidant or a diet low in selenium. All this could be influencing the expression of symptoms associated with the disorder.

Keywords: Essentials minerals, Selenium, ASD, ICP-MS

SELENIUM AND SELENIUM COMPOUNDS IN MARINE ORGANISMS

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Norway Selenium content and its chemical species in plant and animal organisms varies and depend on the environmental conditions to which the animal/plant is exposed. Most of Se in mussel and fish tissues has been associated with organic forms such as selenomethionine. Other Se compounds such as trimethylselenonium has been detected in oysters, mussels, and trout. Recently, a novel selenium-containing compound, 2-selenyl-N_α,N_α,N_α-trimethyl-L-histidine (selenoneine) [1] , was identified in blood and other tissues of bluefin tuna, and in muscle of various fishes. In this study, we investigate the quantity and type of Se compounds in zooplankton samples: scallop, calanus *Calanus finmarchicus, Euphasia superb* and shelfish samples: blue mussel, oyster and *c*ommon crab, collected form from various locations along the Norwegian coastline. The highest Se concentration was determined in the blue mussel sample, this sample also exhibited large variation in selenium content. The difference between the lowest and the highest concentration was 6.78 mg Se kg^{-1} dm (maximum 8.23; mean 3.50; SD 1.21 mg Se kg^{-1} dry mass; (n = 67)). Soluble compounds smaller then 3k MW were easily extracted by the phosphate buffer and accounted for around 40% of total selenium in both sea plankton and shellfish samples. LC-ICP-MS analysis showed that both selenate and selenite were found in extracts from sea plankton and shellfish samples. There were no differences in the content of Se(IV) and Se(VI) in relation to total Se concentration for analysed samples. The content of selenomethionine in sea plankton and shellfish samples, was much lower than usually observed in fish samples (41.5 and 98.4%) and represented only 12% of the Se detected in extracts of common crab claws. The chromatograms of protease hydrolysates of blue mussel revealed the presence of at least eight Se compounds eluted at retention times corresponding to organic compounds.

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JJ BERZELIUS (1779–1848) A LOOK AT HIS LIFE AND LABORATORY

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Jöns Jacob Berzelius is perhaps the greatest Swedish chemist, as well as a physician of his day. He developed the modern technique of 2 symbol chemical formula notation, i.e.; Se, used throughout the Periodic Table of today. He developed the notation for water, H2O; and he introduced to the literature the words, catalysis, isomer, polymer and protein. He became Professor of Chemistry and Pharmacology at the Karolinska in 1807 and is together with John Dalton, Antoine Lavoisier, and Robert Boyle considered one of the initial Fathers of Modern Chemistry as well as the Father of Angloamerican (chemistry based) Medicine. Between 1818 following his discovery of Selenium at Gripsholm, where he partly owned a gun powder (S) factory at Mariefred, he became Secretary of the Academy of Sweden until his death in 1848. It is because of Berzelius that we are here in Berlin in 2013 for The 10th International Meeting on Selenium and that The 11th International Symposium on Selenium should be held in Stockholm, Sweden to commemorate the Bicentennial Publication of the Discovery of Selenium by Berzelius in 1817. This poster shows some of the laboratory equipment, chemicals, actually hand made and used by Berzelius. It is but a small glimpse into his genius, his laboratory and illustrious scientific life. Born: August 20, 1779, Linköping, Sweden Died: August 7, 1848, Stockholm, Sweden Discovered: Silicon, Selenium, Cerium and Thorium Over 250 Scientific Papers and Books; "The Use of the Blowpipe in Chemical Analysis"; "The Examination of Minerals"; "Lectures in Animal Chemistry" (Medical Biochemistry) Education: MD. Uppsala University, Katedralskolan, Linköping. Awards: Copley Medal, French Honary Medal, Nobleman by Swedish King. Most photographs of the Berzelius Exhibition by Mallory Boylan with Permission for non-commercial display; Stockholm 2011.