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INTERNATIONAL SYMPOSIUM ON TRACE ELEMENTS IN MAN AND ANIMALS (TEMA) 12 – SCIENTIFIC DEVELOPMENTS, NOVEL APPLICATIONS AND PROGRESS INTO THE 21ST CENTURY

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The study of trace element metabolism and the function of these elements within the body (both human and animal) is as challenging today as it has ever been. Advances in molecular biology, genomics, transcriptomics and proteomics have started to answer many fundamental questions, but at the same time are raising many more. TEMA symposia bring together experts in all areas of trace element research to present and actively discuss the role of trace elements in health and disease in animals and humans.

TEMA meetings have fundamentally two main objectives: firstly to highlight work at the cutting edge of trace element research by capturing recent progress on the functional role and metabolism of trace elements and secondly to establish a forum for the free exchange of ideas among scientists investigating fundamental aspects of trace elements in the field of nutrition, human or veterinary medicine, agriculture, biology, ecology and the protection of the environment.

TEMA12 successfully attempted to move forward the area of trace element metabolism into the ever-expanding 'omics' era. Plenary sessions in transcriptomics and metalloproteomics and breakout sessions in metabolomics and knockout models were introduced alongside the more traditional topics of trace element problems in developing countries and pre- and post-natal trace element nutrition. This approach allowed scientists, some without a background in trace element research, to provide novel approaches to both the metabolism of trace elements and also the interpretation of experimental data.

Four workshops, two specifically for copper and selenium and two more general in the areas of neurodevelopment and trace elements and interactions amongst trace elements provided much lively discussion as did a debate on the pros and cons of iron supplementation.

The setting for TEMA12 was the spectacular Antrim coast in Northern Ireland near the world heritage recognised Giant's Causeway – the inspiration for the TEMA12 logo. The conference attracted 250 delegates from 27 countries representing all of the continents. The abstracts of talks presented by invited speakers and oral and poster communications by delegates at TEMA12 follow.

ZINC TRANSPORTER EXPRESSION AND THE HYPOZINCEMIA OF INFLAMMATION AND INFECTION

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Reductions of plasma zinc concentrations accompany insults such as acute inflammation or infection. Metabolic analyses have shown that the liver accumulates extra zinc during this redistribution process. To investigate how hepatic zinc metabolism is altered during these conditions, expression of 14 zinc transporter genes from both zinc transporter gene families was evaluated using quantitative PCR, immunocytochemistry, and fluorometric analysis of Zn^{2+} transport. In mice with turpentine-induced inflammation, the most significant change in transporter transcript abundance was for Zip14, with a > 3 fold increase in wild-type mice in response to the inflammation. This increase was accompanied by significant hypozincemia. IL-6 knockout mice did not upregulate Zip14 or exhibit reduced plasma zinc levels in response to the acute inflammation. Treatment of wild-type mouse hepatocytes with IL-6 in culture upregulates Zip14 mRNA and Zn^{2+} accumulation. Using an antibody to an extracellular epitope of Zip14 as defined by an analysis of topology, fluorescence microscopy showed IL-6 produced localization of Zip14 protein to the plasma membrane. Incubation of the hepatocytes with that antibody lowered Zn^{2+} accumulation. Transfection of HEK cells with Zip14 cDNA increased ^{65}Zn accumulation, and increased abundance of Zip14 localized to the plasma membrane. We conclude that, since Zip14 functions as a plasma membrane localized importer of Zn^{2+}, its regulation by IL-6 is a major factor responsible for the hypozincemia of inflammation.

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PALEOLITHIC DIETS – EVOLUTIONARY ASPECTS OF DIETARY TRACE ELEMENTS

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The genetic code behind the trace element requirements of humans was designed during several million years of evolutionary adaptation to the available food items. After the appearance of fully modern humans around 150,000 years ago, the human genome has apparently changed very little, considering the close physiological resemblance between different ethnic groups. During the Paleolithic (2.5 million – 10,000 years BP), the genus Homo subsisted om hunting and gathering; fruit, leafy vegetables, roots, meat, organ meats, fish, shellfish, insects, eggs and nuts were staple foods in varying proportions. A large number of edible plant species was available. Seeds and beans were not consumed in large quantities and were rarely or never obtained from the same plant species throughout the year. After the emergence of agriculture, less than 10,000 years ago, food habits changed markedly. Today, around 70 % of the energy intake is typically provided by foods that were practically unavailable during human evolution, i.e. cereals, legumes, dairy products, refined sugar, fats and oils. Energy-dense western food items are low in trace elements, mainly because these nutrients are virtually absent in refined sugar, fats and oils. The relatively high fat content of domestic meat and meat products, compared with meat from wild animals, further decreases trace element content in modern foods. Iron status of paleolithic man was probably better than that of 21th century Europeans. Iron bioavailability was enhanced by ascorbic acid in fruit and vegetables and by the low intake of iron-chelating phytates from seeds and beans. When meat or fish were abundant, iron intake was particularly high, along with zinc and selenium. The low phytate intake also increased the bioavailability of zinc. The content of copper, manganese and chromium was generally high in paleolithic diets, although a prudent western diet including whole-grain cereals may often provide similar amounts of these trace elements. However, it should be noted that cow's milk is typically very low in copper and manganese. Since iodized salt and dairy products were not available to our paleolithic ancestors, only those with high regular access to fish or shellfish would be expected to have reached the currently recommended intake of iodine. There is insufficient data to suggest that humans, by way of natural selection, would have become completely dependent on marine food sources. Therefore, it is highly possible that human requirements for idodine are currently increased by some lifestyle factors. These theoretically include goitrogens in certain roots, vegetables, beans and seeds. Other comparisons between paleolithic and current western diets, that are relevant to trace element nutrition, are more uncertain. The content of molybdenum and fluorine varied depending on ecological niche but was probably not very different from current diets. A few published studies suggest that wild plants are richer in Fe, Zn and Cu than their cultivated counterparts. In summary, it is suggested that paleolithic diets provide a reference standard for optimal human nutrition. This could have bearing on the requirement, intake and bioavailability of several trace elements.

NUTRIGENOMICS AS A TOOL FOR UNDERSTANDING THE EFFECTS OF MINERAL SUPPLEMENTATION ON ANIMAL HEALTH AND METABOLISM.

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The completion of the human genome project in 2003 has led to dramatic changes in the way we view and study biological processes. Technologies that allow global evaluation of the effects of mineral supplementation strategies on gene expression are now available to quantitatively describe the effects of specific nutrients at the molecular level. Nutrigenomic approaches using oligonucleotide microarrays have established that dietary selenomethionine can significantly influence gene expression in mouse intestinal tissues by upregulating genes associated with cellular stress and cell cycling, and by downregulating genes associated with selenoprotein production, lipid transport and detoxification mechanisms (Rao et al. 2001). More recent studies of gene expression in mice have shown that more than 2500 transcripts (11% of a 23,000 gene array) were influenced by various selenium supplementation strategies. However, strategies using selenomethionine, sodium selenite and Sel-Plex (selenium yeast) each resulted in different patterns of gene expression, indicating that the chemical form of the selenium has key effects on the role of selenium in regulating metabolism. Such studies are rapidly changing the way we view mineral supplementation strategies.

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TRANSCRIPTOMICS: THE USE OF NEW TECHNOLOGIES TO COMPLEMENT TRADITIONAL APPROACHES

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The development of microarray technology has transformed the scope for exploration of complex interactions between dietary components and gene expression in human cells and tissues. Using this technology it is possible, within a single experiment, to obtain an overview of effects on gene transcript profiles at the partial or even the entire genome level. The last few years have seen a growing trend to exploit microarrays in nutrition research paralleled by a dramatic increase in discovery of genes that can be regulated by the diet. While lists of up- and down-regulated genes commonly represent starting points for further investigations and hypothesis development, any researcher who has performed such a study will be well aware that the one key challenge is to sift through the often bewildering range of candidate genes identified. However, buried in the data obtained from appropriately designed microarray studies is information which may be indicative of co-regulation of genes and regulatory mechanisms. Extraction of this information provides the means to narrow down subsequent avenues of investigation and enhance progress. The power of this technology will be illustrated using examples of studies in cell culture systems and animal models that have made use of microarrays to investigate molecular mechanisms involved in trace element homeostasis and metabolism. The scope for future transcriptomic studies involving human volunteers will be discussed and current initiatives to promote best practice and standardisation in nutritional microarray study design, execution and reporting will be reviewed.

TRANSCRIPTOMICS IN THE LIGHT OF GENOMICS: WHAT WE KNOW AND WHAT WE DON'T KNOW

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Microarray technology has made it possible to analyse the very complex effects on gene transcription, and mRNA degradation, of a multitude of physiological factors, including nutritional states. The sophisticated techniques needed to detect meaningful patterns of transcriptional control in response to physiological changes, and to cope with the alarming diversity of gene expression in outbred human experimental subjects, have been fully treated elsewhere. The results of microarray expression analysis make sense in terms of pre-genomic concepts. The transcriptional activities of genes encoding mRNAs are controlled by transcription factors, complexes of proteins that recognise specific sequences at promoter sites immediately upstream of the genes, bind to them and recruit RNA polymerases. This is true as far as it goes. Reality is, however, more complex. As well as promoters, there are other sites in the DNA that profoundly increase or prevent transcription of neighbouring genes: enhancer and repressor elements, that do not have to be close to the genes they regulate, and need not even be on the same chromosome, and insulator elements that can block enhancers from affecting genes too far away. With the use of ChIP-chip techniques (chromatin immunoprecipitation, followed by microarray analysis of the precipitate) a picture is emerging of transcriptional control by long-distance interaction between regulatory sequences, with manipulatable loops of chromatin bringing enhancers and promoters together, and with these interactions producing large-scale epigenetic changes in chromatin structure. At the same time, analysis with whole-chromosome microarrays is revealing that the genome contains much "dark matter", sequences not previously identified as genetic but nevertheless transcribed; and comparative genomics is revealing conserved, even hyperconserved, regions of DNA with no coding function. Some of these newly-discovered regions probably correspond to non-coding RNAs that are involved in determining or maintaining chromatin structure, and hence transcriptional control of coding genes.

KNOCKOUT OF THE SELENOPROTEINS PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE, CYTOSOLIC THIOREDOXIN REDUCTASE, AND MITOCHONDRIAL THIOREDOXIN REDUCTASE REVEAL IMPORTANT DEVELOPMENTAL FUNCTIONS.

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Selenium, as integral part of selenoproteins, plays a crucial role in the maintenance of cellular redox homeostasis. To study the function of important cellular anti-oxidative redox-active enzyme systems, we generated mice deficient in the selenoproteins phospholipid hydroperoxide glutathione peroxidase (PHGPx/GPx4), cytosolic thioredoxin reductase (Txnrd1) and mitochondrial thioredoxin reductase (Txnrd2). Homozygous embryos of these three knockouts were lethal at distinct stages of development. PHGPx knock-out embryos died at embryonic day (E)7.5, Txnrd1 embryos between E8.5 and E10 and Txnrd2 embryos at day E13.5. Whereas the reason for embryonic death of PHGPx knockouts shortly after implantation is not yet understood, Txnrd1 knockouts showed impaired growth and developmental retardation and Txnrd2 knockouts were characterized by disturbed haematopoiesis and malformation of the heart. Despite the severe phenotype of homozygous knockouts, loss of function of one allele of the three knockouts did not develop detectable phenotypes. Our results indicate important functions of the three selenoproteins PHGPx/GPx4, Txnrd1and Txnrd2 in mouse development. However, one copy of these selenoprotein genes appears sufficient to fully maintain biological functions. These findings open new approaches to understand the essentiality of the trace element selenium.

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METALLOTHIONEIN/ZINC CHANGES AND BIRTH DEFECTS IN MICE FOLLOWING ENDOTOXIN, ALCOHOL AND DEXAMETHASONE TREAMENT IN EARLY PREGNANCY.

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Infection, stress and consumption of alcohol in early pregnancy are all linked with poor fetal outcome. The aim of this study was to compare the effects of endotoxin (LPS), dexamethasone (DEX), and ethanol (ETOH) on disturbances in plasma Zn (pZn) and fetal outcome in C57BL6 mice (wild type and MT I and II knockout). Temporal changes in MT and Zn were first examined in wild type mice in the first 24h following exposure to LPS (2mg/kg ip), ETOH (2.9g/Kg ip at 0 and 4h) and DEX (8mg Kg ip) . The order of MT induction was LPS>ETOH>DEX and this correlated with the decrease in pZn (endotoxin 86% decrease, 8h; ethanol 70% decrease at 8h, dexamethasone 30% decrease at 12h). None of the treatments decreased pZn in MT knockout mice. When administered at gestational day 8 to wild type mice, fetal abnormalities correlated with the extent of change in pZn. Fetuses from MT knockout dams were not significantly affected by any treatment. Zn treatment (2mg/Kg sc) at the time of administering the teratogens completely prevented birth abnormalities. Disturbances in Zn metabolism via the inappropriate induction of maternal hepatic MT provides a link between these teratogens.

DOUBLE KNOCKOUT OF CU, ZN-SUPEROXIDE DISMUTASE (SOD1) AND SELENIUM-DEPENDENT GLUTATHIONE PEROXIDASE-1 (GPX1) PROTECTS AGAINST ACETAMINOPHEN TOXICITY

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Acetaminophen (APAP), a widely used analgesic-antipyretic drug, induces glutathione depletion, generation of reactive nitrogen species (RNS), and other toxicities in liver. The objective of our study was to determine the impact of knockout of SOD1 and GPX1 alone or together on APAP-induced hepatotoxicity. In Experiment I, wild type (WT), $GPX1^{-/-}$, $SOD1^{-/-}$, and $GPX1^{-/-}SOD1^{-/-}$ (n = 6-12 per group) mice were given an ip injection of 600 mg APAP/kg of body weight. While all $SOD1^{-/-}$ and $GPX1^{-/-}SOD1^{-/-}$ mice survived the entire 72 h observation period, 75% of the WT and $GPX1^{-/-}$ mice died within 20 h. In Experiment II, all four genotypes (n = 10 per group) were injected with APAP (300 mg/kg) or saline, and killed 5 h post-injection. The APAP treatment resulted in a sharp rise ($P < 0.05$) in plasma alanine aminotransferase (ALT) activity and significant ($P < 0.05$) depletion of hepatic glutathione, as compared with their saline-injected controls. In contrast, the APAP-treated $SOD1^{-/-}$ and $GPX1^{-/-}SOD1^{-/-}$ mice had little rise in plasma ALT activity and much less hepatic glutathione depletion. Western blot analysis and immunostaining indicated hepatic protein nitration induced by APAP in the WT and GPX1-/- mice, but not in SOD1-/- or GPX1-/- SOD1-/- mice. In conclusion, the APAP-induced lethality and oxidative injuries were abolished by knockout of SOD1. In conclusion, knockout of SOD1 alone or with GPX1 protects mice against the APAP-induced lethality and oxidative injury in liver. Our results suggest intriguing metabolic roles of the two major intracellular antioxidant enzymes in APAP-induced oxidative stress and toxicity.
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BIOLOGICAL ROLES OF SELENOPROTEINS FOR BRAIN DEVELOPMENT AND FUNCTION. NOVEL AND UNEXPECTED FINDINGS USING TRANSGENIC MOUSE MODELS

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Selenium (Se) is an essential trace element as established through studies feeding Se deficient diets to animals. However, without further insults, spontanous neurological deficits in such rodents were never established. Targeted disruption of the gene for selenoprotein P (SePP) in mice revealed neurological deficits involving seizures and ataxia. Unlike in rodents fed a Se deficent diet, Se content and selenoenzyme activities were substantially reduced in SePP knockout (KO) mice. Rescue of the neurological phenotype by dietary Se supplementation of SePP KO mice suggested a Se transport role of SePP. However, using another mouse model, we have recently shown that SePP secreted by the liver is a major determinant of kidney, but not brain Se supply, indicating that SePP expressed in the brain might play a role in Se accumulation and/or trafficking in the brain. Nevertheless, it is still not established which selenoproteins are required for brain development and function. To address this question we have established mice with neuron-specific inactivation of selenocysteine tRNA[Ser]Sec, thus abolishing neuronal selenoprotein biosynthesis. Resulting KO mice show a devastating neurological condition and die before the end of their second week of life. Among other phenotypes, a striking derangement of cerebellar development is observed.

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DO SINGLE NUCLEOTIDE POLYMORPHISMS IN SELENOPROTEIN GENES AFFECT THE RISK OF PROSTATE CANCER?

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While evidence appears to be growing that low selenium (Se) status increases the risk of prostate cancer, it is not known whether this applies to all men or only to a sub-population. The efficiency with which individuals can use Se to form selenoproteins varies with the occurence of single nucleotide polymorphisms (SNPs) in their selenoprotein genes such that some individuals may have a higher requirement for Se than others. SNPs occur in genes for a number of selenoproteins that have been linked to cancer risk, e.g. Leu/Pro198 in the cytosolic glutathione peroxidase selenoprotein (GPx1); T/C718 in phospholipid glutathione peroxidase (GPx4); 811C/T and 1125G/A in Sep15. We have a unique set of samples from Sweden, an area of relatively low Se status: DNA from 1400 prostate cancer cases and 800 cancer-free male controls matched for age and location. Clinical, demographic and other information is available on the subjects. We are genotyping these samples for functional selenoprotein SNPs. Se concentration in stored plasma is also being measured. Thus far, we have found no difference in the frequency of alleles or genotype distribution of the Sep 15 811C/T polymorphism between prostate cancer cases and controls. An association between risk of prostate cancer and selenoprotein SNP genotype would mean that sub-groups of men, identified by their selenoprotein SNPs, could be advised to increase their Se intake in order to increase the expression level of protective selenoproteins.

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USE OF STABLE ISOTOPES AND MATHEMATICAL MODELLING TO INVESTIGATE HUMAN MINERAL METABOLISM

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Generating information on how minerals are metabolised and distributed in humans is difficult because invasive techniques can rarely be used. Orally or intravenously administered stable isotope-enriched sources of most inorganic nutrients can easily be detected in blood, urine and faecal samples of volunteers using mass spectrometric methods. When the data are analysed using mathematical modelling, it is possible to quantify minerals from both endogenous and dietary origin, and calculate the kinetics. Most modelling techniques used in nutrition were originally developed for drug metabolism and are collectively referred to as pharmacokinetics. This talk will focus on the application of pharmacokinetic modelling to study copper, iron, zinc and selenium metabolism.

DECIPHERING THE IRON ISOTOPE MESSAGE OF THE HUMAN BODY

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The relative abundance levels of stable isotopes in nature are no natural constants. Transport processes can alter the isotopic composition of an element if transport is mass sensitive and not quantitative. UsingMulticollector-Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS), we discovered recently that all iron in the biosphere, incl. the human body, is depleted in the heavier iron isotopes (Walczyk & von Blanckenburg, 2002). In addition, each individual bears a distinct iron isotopic signature in blood with females having on average more of the heavier iron isotopes in blood than males. A pilot survey in human tissue and dietary iron sources indicated that the iron isotope effect in blood is primarily determined by preferential absorption of lighter iron isotopes by the body. Our recent survey in haemochromatosis patients suffering from iron overload due to permanent failure of regulatory mechanisms of iron absorption has confirmed this hypothesis (Krayenbühl et al. 2005). In a parallel survey we could also show that iron in liver differs from iron in blood and muscle tissue in its isotopic composition (Walczyk & von Blanckenburg, 2005). Based on available data, possible mechanisms for inducing an iron isotope effect at the cellular and molecular level during iron uptake are presented. The potential of iron isotope effects in human blood as a long-term measure of dietary iron absorption is discussed. Our earlier hypothesis that the isotopic biosignature in blood is stable over at least one year has been confirmed recently (Ohno et al. 2004). This may open the possibility to elucidate the largely unknown role of genetic predisposition in the etiology of iron deficiency and iron overload.

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URINARY SELENIUM METABOLITES, SELENOSUGAR AND TRIMETHYLSELENONIUM

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Diverse selenium (Se) compounds are the nutritional source of Se used for seleno-enzyme synthesis in mammalian tissues. Excreted Se, however, is found primarily in urine in two forms: 1ß-methylseleno-N-acetyl-D-galactosamine (selenosugar) and trimethylselenonium (TMSe). Selenosugar is thought to be the major metabolite generated under conditions of Se availability up to the lowest toxic level. When higher, toxic Se doses are applied, TMSe is excreted. Little is known of the events that govern which excreted Se metabolite is formed under different exposure conditions. We have recently shown that selenosugar is a major metabolite under all exposure conditions (Suzuki et al, 2005). TMSe, on the other hand, is excreted in response to higher toxic doses in young, and little in adult, rats. Thus, TMSe appears to be unreliable as a biological marker for excessive and/or toxic doses of Se and, furthermore, the sugar moiety of selenosugar is supplied from endogenous sources with sugar availability varying with the age of the animal. We show here that exposure to excess Se resulted in excretion of Se into urine in both metabolite forms, with different ratios in the source of Se for each, i.e., Se of selenosugar and TMSe were different in endogenous/exogenous Se ratios depending on dose and observation period. This was observed after feeding isotope-enriched Se compounds to rats, followed by measuring and speciating urinary Se metabolites according to isotope content.

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LONG-TERM MODERATE ZINC SUPPLEMENTATION INCREASES EXCHANGEABLE ZINC POOL MASSES IN LATE MIDDLE-AGED SUBJECTS : THE ZENITH STUDY

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Background: Zinc supplementation may be beneficial for health, notably in the elderly. Assessing the exchangeable Zn pools may be a useful approach for evaluating Zn status. Objective: We undertook this study to evaluate the long-term supplementation effects of two moderate doses of Zn on the mass of the exchangeable zinc pools. Design: three groups of healthy late middle-aged male subjects (n = 16/group) participated in a stable isotope Zn kinetic study following 6 months of daily supplementation with placebo (0 mg) or 15 mg or 30 mg of supplemental Zn. At the end of the supplementation period, each subject received an intravenous injection of 1 mg of ^{70}Zn, and the plasma Zn disappearance curve was monitored for the next 10 d. Two approaches have been applied to determine the characteristics of exchangeable Zn pools: 1) formal tri-compartmental modeling, and 2) a simplified determination of the total mass of the rapidly exchangeable Zn pool (EZP). Results: The exchangeable Zn pool masses for the three considered pools were as follows : 2.15, 12.7 and 100.5 mg Zn. Rapidly exchangeable Zn pool mass was 143 mg Zn. Zn supplementation significantly increased exchangeable Zn pool masses regardless of the approach employed to determine these pools. In addition, these data confirmed that Zn exchangeable pool masses correlate positively with zinc dietary intake and correlate negatively with subject age. Conclusions: our data demonstrated that a long-term supplementation with two moderate doses of Zn is efficient to increase exchangeable Zn pool masses in late middle-aged subjects.

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APPLICATION OF SATURATION KINETIC MODELING IN EVALUATION OF RELATIONSHIPS BETWEEN QUANTITIES OF INGESTED AND ABSORBED ZINC

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Mammalian absorption of zinc is primarily through the enterocytes of the upper small intestine and involves a saturable active transport process across the apical membrane. The objective of this study was to examine the dose-response relationship between the quantity of zinc absorbed and the quantity ingested from meals applying saturation kinetic modeling to two human data sets that have sufficient data points for this purpose. One of these is the data set used in 2001 by the Food & Nutrition Board in the USA to estimate DRIs for young adult men. The other is for Peruvian children age 4 yrs fed wheat-based test meals at breakfast and lunch with three levels of zinc fortification (López de Romaña, et al, 2005 AJCN, in press]. For each of these data sets, estimated physiologic requirements for these population groups were approximately 50-60% of estimated maximal absorption. The efficiency of utilization of increments in ingested zinc beyond this percentage was low in contrast to utilization of zinc in quantities less than those required to achieve 50% maximal absorption. Among the conclusions to be derived from this modeling is that metabolic evaluation of the efficacy of any intervention designed to prevent zinc deficiency requires baseline knowledge of the quantity of zinc absorbed in relation to physiologic requirements. *Supported in part by the Thrasher Research Fund.*

COPPER TURNOVER AND RETENTION IN HUMANS WITH INCREASING COPPER INTAKE

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We infused stable isotopes of copper into adult men in a number of studies with copper intakes ranging from 0.38 to 7.8 mg/d to determine the effect of copper intake on turnover. Infusions were done after at least 6 days of adaptation to the copper intake. All stools and urine were collected for 12 days following the infusion. The fractions of the isotope doses excreted into the gastrointestinal tract and eliminated in the stools were determined by mass spectrometry. The percent of the infused dose eliminated in 6d was 6.9% when intake was 0.38 mg/d. It increased as intake increased, reaching 30% when intake was 7.8 mg/d. After 12d, 12% of the dose was eliminated at the lowest intake and 46% at the highest. Total endogenous losses also increased as intake increased. Because copper turnover is slow when intake is low and increases markedly as intake increases, copper balance can be maintained over a range of intakes, but tended to become negative when intake was less that 0.5 mg/d. Copper was consistently retained when intake reached 2 mg/d and increased further with increases in copper intake. Retention ranged from 0.3 to 0.9 mg/d. This is not a problem with usual dietary patterns, but the data suggest that routine use of dietary copper supplements containing 2 mg or more per day could lead to accumulation of excess copper.

MATERNAL IRON DEFICIENCY DURING PREGNANCY INDUCES SYNDROME X IN THE OFFSPRING IN RATS

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There are increasing data that maternal nutritional imbalance during pregnancy results in deleterious changes in the offspring as adults. These observations have been codified as the “fetal origins” hypothesis. We

have developed a model using maternal Fe deficiency to examine the mechanisms underpinning the observations. Maternal rats were fed Fe deficient diets for 4 weeks prior to mating and throughout mating. Animals were either killed at different stages during gestation or allowed to give birth. The offspring were cross fostered onto normal dams and fed normal diet throughout life. Despite having normal weights, the offspring of dams fed Fe deficient diets had high blood pressure, and higher fat content than those born to normal controls. We identified critical periods during gestation when the animals were most sensitive to changes in cardiovascular function and also periods when growth was most effectively compromised. The data show clearly that, as has recently been regcognised in humans, maternal Fe status is critically important for normal growth and development, and alterations can lead to permanent harm in the offspring.

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EFFECTS OF DIETARY ZINC AVAILABILITY ON PLACENTAL ZINC TRANSPORT IN THE MOUSE, AND ITS POTENTIAL IMPLICATIONS FOR THE FOETUS

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To determine if the regulation of placental zinc transport may contribute to foetal zinc homeostasis, we examined the response of placental zinc transporters to changes in dietary zinc intake in the mouse. Mice (n = 6-12) were fed diets containing 15 mg Zn/kg (marginally zinc-deficient; ZnD), 50 mg Zn/kg (zinc-adequate; ZnA) or 150 mg Zn/kg (zinc supplemented; ZnS) from the onset of pregnancy until day 17. Analysis of placental tissue by RT-PCR showed that, with the exception of ZnT5 in mice fed the ZnD diet, levels of all zinc transporter mRNAs studied (ZnT1, ZnT4, ZnT5, ZIP1) were reduced in mice fed both the ZnD and ZnS diets compared with the ZnA diet. This pattern was confirmed by immunoblotting for ZnT1 and ZnT5. Foetal weight in mice fed the ZnD diet (0.64 ± 0.01 g, mean ± SEM) was significantly lower ($P < 001$) than in the other groups (ZnA: 0.71 ± 0.02 g; ZnS: 0.76 ± 0.03 g), indicating that gene regulatory responses to changes in dietary zinc are insufficient to maintain adequate zinc nutrition even under conditions of moderate zinc deficiency. Moderate zinc deficiency during pregnancy, therefore, may affect the long-term health of the neonate.

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DIETARY SELENIUM SUPPLEMENTATION IN LAYER-TYPE CHICKENS: EFFECTS ON SE CONTENT OF EGGS, FERTILITY AND EMBRYO SURVIVAL

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A complex antioxidant system including Se-dependent enzymes (ex: GSH-Px) is present in the sperm storage tubules of chicken hens to protect sperm plasma membranes against lipid peroxydation (Brèque et al, 2003). In a series of experiments in which chicken hens were supplemented with Se, we tested the effects of female age (mature and old) and source of Se (Selenite, batch 030301 and Sel-Plex, batch ES-716) at various concentrations (from 0.10 to 0.46 ppm) on feed consumption, body weight, laying performance, antioxidant status of eggs (albumen and yolk), fertility and embryo mortality. Se supplementation had no detectable effect on feed consumption, body weights and rate of lay at any of the doses tested irrespective of the source. Accordingly, Se content in albumen and yolk was proportional to the degree of Se dietary supplementation at both ages tested but Sel-plex was found more efficient than Selenite irrespective of concentration and age. Finally, a favourable effect of Se dietary supplementation on fertility and embryo mortality was detected at one occasion in aging hens. Based on previous observations by these and other authors, it is postulated that a beneficial effect of Se on fertility observed in naturally mated breeder flocks originates from a better overall quality of sperm in Se–treated males rather than from female Se supplementation, at least during the earliest period of the season.

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SUPPLEMENTATION OF AVIAN MATERNAL NUTRITION WITH ORGANO-SELENIUM COMPOUNDS AND POLYUNSATURATED FATTY ACIDS AFFECTS THE EMBRYONIC MORTALITY DURING INCUBATION

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Young broiler flocks are known to produce eggs that have a significantly lower hatchability when compared with eggs from older birds, possibly due to an altered fatty acid profile in the egg (Noble & Yafei, 1988). Broiler breeders (N = 353) were fed either diets low in selenium (0.1 mg/kg) with two oil sources: soya (SO) or fish oil (FO) and the same diets with added selenium (0.5 mg/kg) as Sel-Plex® (SO+Se, FO+Se). Eggs were collected at 22 and 27 wks of age and incubated. Candling and a breakout analysis of embryos that died was performed to assess embryonic mortality. Mortality during the first and second week of incubation was not affected by dietary treatments, while mortality in week 3 was significantly ($P < 0.05$) greater in the FO and FO+Se treatments compared to SO, irrespective of parent age. By 27 weeks of age, mortality was significantly lower in the FO+Se treatment compared to the FO treatment, which was taken to be indicative of the beneficial effects of selenium. Noble RC & Yafei N (1988) An association between low embryo hatchability in eggs from young broiler birds and aspects of lipid metabolism. In: Proceedings of the XVIII World's Poultry Congress Nagoya, Japan 640-641.

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DIETARY ZINC SUPPLEMENTATION LIMITS ENDOTOXIN-INDUCED TERATOGENICITY IN MICE

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Infection during the first trimester of pregnancy has been associated with spontaneous abortion, pre-term delivery and congenital abnormalities. Previous studies (Carey et al 2003) indicated that subcutaneous injection of Zn prevents endotoxin (LPS)-induced teratogenicity. The aim of this study was to examine whether increasing or decreasing dietary Zn also alters the teratogenic effects of LPS. Female C57BL6 mice were mated and placed on diets containing 5, 35 or 100 ppm Zn. On gestational day (GD) 8 pregnant dams were injected with either LPS (0.5 mg/kg sc) or saline and killed on GD18. In fetuses from saline injected dams, increasing or decreasing dietary Zn did not alter the abnormalities per litter. With LPS treatment, the number of abnormalities per litter was significantly higher in mice fed 5 & 35 ppm Zn diet (3- & 2-fold saline controls, respectively) whereas the rates in dams on the LPS +100 ppm Zn were not significantly different from the saline control groups. The preventative and potentially beneficial effect of zinc was also reflected in the greater size of fetuses (weight and length) from the LPS +100 ppm Zn treatment group.

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AN OBSERVATIONAL STUDY OF A COHORT OF PRIMIPAROUS WOMEN OF HIGH SOCIO-ECONOMIC STATUS AND THEIR PREGNANCY OUTCOMES

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Associations between maternal dietary trace element intake during pregnancy and lactation on pregnancy outcomes were studied. Total intake of trace elements from food and supplements exceeded the recommended dietary intake for Fe, Zn, Mn, Cu, I and Se at 26 and 34 weeks of pregnancy. Zn and Se intake provided 80% and 71% respectively of the recommended daily intake during lactation. Gestational Se intake was significantly related to maternal Se levels ($P = 0.021$). Neonatal Se levels were related to maternal Cu intake during pregnancy ($P = 0.002$). Colostrum Cu levels were significantly related to Se intake during pregnancy ($P = 0.01$). Fe and Zn levels in breast milk were significantly related to gestational intake of Mn, Zn, I and Fe ($P < 0.005$). Trace element intake was adequate during pregnancy and lactation for all trace elements. The use of dietary supplements increased intakes for all trace elements above the recommended amounts except for Se during pregnancy. This had no adverse consequences for maternal or neonatal health. The reduced use of dietary supplements during lactation coupled with the increase in the recommended amounts for Zn and Se suggests that 20 to 30 % of subjects in this study had inadequate intakes during lactation.

METABOLOMICS IN HUMAN NUTRITION

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Metabolomics, the simultaneous measure of all or a very large fraction of the metabolites in a biofluid, has made significant progress in pharmacology and toxicology but has yet to make an impact in human nutrition. There are several reasons why the application of metabolomics to human nutrition will be more problematic than its application to medicine and toxicology. In the former, powerful metabolic signals (inflammatory, endocrine, vascular, neural and so on) emanate from sites of pathological damage and these metabolites and signalling compounds play a significant role in distinguishing between different groups of patients and controls. Equally, in both pharmacology and toxicology, the input is a single entity, often administered in reasonably high doses, which have a direct effect on a specific metabolic pathway. In contrast, in human nutrition, the metabolic effects of nutrients at normal levels of intake constitute a much weaker input and significantly, the input of nutritional signals will be confounded by the signals from the myriad of non-nutrient phytochemicals in foods and from non-nutrient compounds produced when food is cooked. Since these compounds are "xenobiotics" they will exist in high concentrations in urine relative to the urinary output of metabolites that arise from nutrient intake. Nonetheless, metabolomics holds great potential for human nutrition not least in its capacity in metabolic profiling with the metabolome as the biomarker and with the use of pattern recognition techniques for clustering different known metabolic profiles. Extending beyond simple pattern recognition techniques to the discovery of the nature of the metabolites which distinguish specific groups, either clinical or dietary, will raise a challenge in terms of metabolite identification. In many instances, the techniques used in metabolomics (NMR and MS) will identify only a fraction of the observed metabolites in biofluids. Metabolomics is high on the NIH roadmap and should receive increasing attention in human nutrition.

APPLICATION OF NMR AND MASS SPECTROMETRY BASED METABOLOMICS TO BIOLOGICAL SYSTEMS

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The term metabolomics was coined to describe the development of approaches which aim to measure all the metabolites that are present within a cell, tissue or organism during a genetic modification or physiological stimulus. The major technologies being used for this process are proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, Gas Chromatography - Mass Spectrometry (GC-MS) and Liquid Chromatography - Mass Spectrometry (LC-MS), in conjunction with pattern recognition approaches. These techniques are high throughput and are cheap on a per sample basis, making them ideal as rapid screening tools. Metabolomics is being used to address a number of issues relevant to toxicology, ecotoxicology, medicine and functional genomics. These applications will be illustrated by work from the author's laboratory examining cadmium and arsenic toxicity in wild mammals (Griffin et al. 2000; 2001), monitoring apoptosis in tumours (Griffin et al. 2003; Lehtimaki et al. 2003) and understanding cardiac disease through metabolomic analysis of cardiac tissue (Jones et al., 2005).

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IDENTIFICATION OF A SINGLE PROMOTER CONTROLLING EXPRESSION OF THE ZN TRANSPORTERS HZTL1 AND ZNT5

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The human Zn transporters hZTL1 and ZnT5 are splice variants of the SLC30A5 gene. ZnT5 has additional exons upstream of the first exon of hZTL1. Treatment of human intestinal Caco-2 cells with 100 μM Zn increased hZTL1 mRNA and decreased ZnT5 mRNA, suggesting the use of alternate promoters. β-galactosidase reporter constructs, comprising regions directly upstream of the first exons of hZTL1 and ZnT5 (hZTL1a: -634bp to +22bp, hZTL1b: -2877bP to +22bp, ZnT5a: -949bp to +45bp, ZnT5b: -2145bp to +45bp) in pBlueTOPO, were transiently transfected into Caco-2 cells. Cells were treated with 3 μM or 100 μM Zn and β-galactosidase activity was measured in cell lysates after 24 h. Statistical analysis was by one-way ANOVA followed by Bonferroni's multiple comparisons test, n = 10-15. The activity of neither hZTL1 upstream region differed from negative control at 3 μM Zn (hZTL1a: 1.00 ± 0.03, Negative: 0.96 ± 0.03; hZTL1b: 1.00 ± 0.01, Negative: 1.04 ± 0.02). Both ZnT5 upstream regions tested showed greater activity than negative control at 3 μM Zn and treatment of the cells with 100 μM Zn caused a reduction in activity of both ZnT5 upstream regions (ZnT5a: Negative: 0.02 ± 0.01, 3 μM = 1.00 ± 0.02, 100 μM = 0.52 ± 0.02, $P < 0.001$; ZnT5b: Negative: 0.02 ± 0.01, 3 μM = 1.00 ± 0.03, 100 μM = 0.49 ± 0.04, $P < 0.001$). These data are consistent with the expression of hZTL1 and ZnT5 from a single promoter upstream of the first exon of ZnT5. Reduced promoter activity appears to underlie the decrease in mRNA levels of ZnT5 seen at 100 μM Zn. Further post-transcriptional control must play a role in the accumulation of hZTL1 mRNA under the same conditions.

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TRANSCRIPTIONAL CONTROL OF GOLGI-DIRECTED ZINC EFFLUX TRANSPORTERS BY ZINC DEFICIENCY

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Intracellular zinc concentration and localization are strictly regulated by two main protein components, metallothioneins and membrane transporters. In mammalian cells, two membrane transporters families are involved in intracellular zinc homeostasis: the uptake transporters called SLC39 or Zip family and the efflux transporters called SLC30 or ZnT family. ZnT proteins are members of the cation diffusion facilitator (CDF) family of metal ion transporters. From genomic databanks analysis, we identified the two novel SLC30 genes, extending the SLC30 family to ten members. We used an expressed sequence tag (EST) data mining strategy to determine the pattern of ZnT genes expression in tissues. *In silico* results obtained for already studied ZnT sequences were confronted with experimental data, previously published. We determined an overall good correlation with expression pattern obtained by RTPCR or immunomethods, particularly for highly tissue specific genes. This method provides a useful tool to complete gene families from sequencing programs and to produce preliminary expression data to select the proper biological samples for laboratory experimentation. Then, we focused on the effect of extracellular zinc status on the regulation of the two trans-Golgi network directed zinc transporters ZnT-5 and ZnT-7 by real-time RT-PCR in zinc supplemented or depleted HeLa cells. We monitored intracellular zinc and compared two fluorophores to assess intracellular zinc status depending on the treatment prior to RT-PCR analysis. While sub-toxic extracellular zinc addition strongly induced the efflux transporter ZnT-1 gene expression, consistent with its activation by the transcription factor MTF-1, zinc-treated HeLa cells did not display any change in ZnT-5 and -7 mRNA levels compared to control cells. In contrast, zinc depletion induced by non-toxic doses of the zinc chelator TPEN resulted in a up to eight fold induction of transporters ZnT-5 and -7 mRNA levels, providing the first evidence of a transcriptional control of these two zinc efflux transporters by zinc deficiency in cultured cells. Our results point out the possible occurrence of a low zinc level transcription factor similar to zap1 but yet to be identified and acting as a zinc-deficiency sensor.

CHROMIUM-L-METHIONINE LOWERS FASTING PLASMA NONESTERIFIED FATTY ACID (NEFA) CONCENTRATIONS IN GROWING PIGS

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Chromium is generally recognized as an essential nutrient for most animal species; however, a dietary requirement for the pig has yet to be quantified. Metabolically, chromium potentiates the action of insulin. Insulin inhibits hormone sensitive lipase and decreases hydrolysis of adipose tissue triglycerides. Therefore, an experiment was conducted to determine the effect of chromium from chromium-L-methionine complex on fasting NEFA concentrations in growing pigs. Eighty-four pigs (19.68 ± 0.214 kg) were grouped by gender, stratified by weight and randomly assigned to pens (n = 20). Pens were randomly assigned to one of four dietary treatments. Dietary treatments were a corn-soybean meal diet supplemented with 0, 0.1, 0.2 or 0.4 mg Cr (as chromium-L-methionine) per kg diet. Diets were fed for ad libitum intake for 33 days. Blood samples were obtained after 16-h without access to feed for later determination of plasma NEFA concentrations. As supplemental dietary chromium level increased, plasma NEFA concentrations decreased (linear effect, P = 0.03). Plasma NEFA concentrations (mEq/L) were 0.18, 0.14, 0.13 and 0.09 for 0, 0.1, 0.2 and 0.4 mg Cr/kg diet, respectively. Chromium-L-methionine is a bioavailable source of supplemental chromium as shown by the observed decrease in circulating NEFA concentrations in growing pigs.

EXCRETION OF A SELENOSUGAR IN HUMAN URINE AFTER INGESTION OF SELENITE OR SELENOMETHIONINE

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Studies on the metabolism of selenium are of interest because of the element's opposing roles as essential trace element and toxicant, in addition to its purported effectiveness as an anti-cancer agent. Since urine is a major excretory route of selenium, urinary selenium metabolites can be used to obtain information about selenium metabolism in the human body. Progress has been made in this area following the recent identification of a selenium-containing galacto-pyranoside (selenosugar 1) as the major selenium urinary metabolite after selenium supplementation. Questions now arise about the effect of the ingested form of selenium and the dose of selenium on the excretion and metabolites. The present study reports the urinary excretion of selenium metabolites after ingestion by a male human volunteer of defined quantities (1.0 mg Se) of selenium as either sodium selenite or L-selenomethionine. Selenium was rapidly excreted with maximum concentrations (ca 500 µg/L) occurring in the first urine sample collected 6 hours after ingestion of the compounds. Selenium concentrations had essentially returned to baseline levels (ca 30 µg/L) within 48 hours, by which time about 40% (selenite) or 30% (L-selenomethionine) of the ingested selenium had been excreted in the urine. Selenosugar 1 was the major metabolite accounting for about 90% of the total urine selenium for both experiments. Preliminary cytotoxicity data for selenosugar 1 will also be presented.

SELENOPROTEINS AND THE HUMAN SELENIUM REQUIREMENT.

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A selenoprotein contains selenocysteine in its primary structure. This rare amino acid is synthesized and inserted into the growing polypeptide chain through a complex process. Twenty-five selenoprotein genes have been identified in the human genome by searches for DNA characteristics that specify selenoprotein production. The selenoproteins serve a number of functions. The selenium in the organism is not distributed equally among the selenoproteins. When selenium is limiting, certain selenoproteins are produced while others are not. Thus, a hierarchy of selenoproteins exists: presumably, ones most important for survival have priority for selenium. The major definition of the selenium requirement states that enough selenium should be consumed to allow full expression of all selenoproteins. Because not all selenoproteins are accessible for measurement, those in blood must serve as surrogates. Plasma glutathione peroxidase (GPX) was used as the surrogate for a study in China in 1983. It was optimized by an intake of 41 µg selenium per day. A later study in New Zealand was interpreted as supporting the Chinese study. Another study was carried out in China recently and it measured both plasma GPX and plasma selenoprotein P. The optimization of GPX activity confirmed the results of the previous studies, but selenoprotein P did not reach its optimum value. This indicates that selenoprotein P is lower in the hierarchy of selenoproteins than plasma GPX and is therefore more representative of all the selenoproteins. Selenium RDAs based on plasma GPX activity will need revision based on selenoprotein P optimization.

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A PROTEOMIC PATH TO TRACE ELEMENT FUNCTION

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Analysis of protein expression patterns, inter-relationships and post-translational modifications has become considerably easier following the development of proteomic techniques. While antibody arrays and protein-protein interaction techniques are examples of emerging specialised methodology for integrating the response of particular proteins and signalling pathways, the basic 2D gel technology provides a global insight into cellular or cell fraction protein expression and modification. The concept of "metallomics", where for example protein spots on gels are interrogated for their metal-binding using laser ablation ICP/MS, requires a new approach to methods of protein preparation and separation in order to preserve metal-binding. At the Rowett Institute, where we have a centralised proteomics facility, we have successfully utilised 2D gel proteomics for the study of protein expression in response to trace element deficiencies. For example, aorta from rats fed a marginally zinc deficient diet for 6 weeks were found to have significantly suppressed protein levels for carbohydrate metabolism enzymes, and this has prompted us to further investigate the role of zinc in regulating insulin activity in vascular tissue. A serious disadvantage of conventional proteomics is that only a fraction of the proteome may be detected on a 2D gel. Strategies for enhancing spot detection and employing appropriate statistical approaches for data analysis are key to exploiting the potential of this technique.

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HFE GENETIC POLYMORPHISMS: INFLUENCE ON IRON STATUS AND MODULATION OF DISEASE RISK.

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Despite rapid advances in nutritional genomics, there are few examples in the area of mineral metabolism of the importance of relatively common genetic polymorphisms in determining nutritional phenotype and their potential to interact with the nutritional environment to modulate disease risk. Hereditary hemochromatosis has been recognized clinically for quite some time and is associated with excessive amounts of absorbed iron, tissue iron overload, organ damage, and increase risk of disease. In 1996, the HFE gene was cloned and a mutation resulting in a tyrosine for cysteine substitution at position 282 (C282Y) was found in the homozygous state to be associated with a large majority of cases of hereditary hemochromatosis in Caucasian populations. The current discussion will focus on an examination of genetic polymorphisms in the HFE gene as determinants of iron status and modulators of disease risk. A significant number of epidemiological studies have investigated the association of genetic polymorphisms (primarily C282Y and H63D HFE mutations) with transferrin saturation and serum ferritin, as markers of iron status, and/or the risk of various diseases. While these studies highlight the potential informative nature of HFE polymorphisms as a determinant of nutritional phenotype, they also illustrate the complex and possible limited usefulness of this genetic information in predicting clinical disease risk.

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MOLECULAR STUDIES OF SELENOPROTEIN GENES: FROM PRIORITISATION TO INDIVIDUAL VARIATION

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Selenium (Se) is essential for optimal human health and supplementation has been reported to reduce cancer incidence. Se is incorporated into selenoproteins (e.g. the glutathione peroxidases) as the amino-acid selenocysteine by a mechanism that requires a specific tRNA and a structure within the 3'untranslated region of the mRNA (3'UTR). During Se deficiency the expression of the various selenoproteins is affected differentially both between different protein and between tissues: in the liver, expression of GPX1 is affected more than GPX4 and in the colon GPX1 and SelW are affected more than GPX2. Experiments with cell lines transfected with reporter constructs linked to different 3'UTRs have shown that such differential effects are partly due to differences in the 3'UTRs of GPX1,2 & 4. Potentially, polymorphisms in the region of selenoprotein genes corresponding to the 3'UTR could influence selenoprotein synthesis and affect the response of individuals to selenium supplementation. Sequencing analysis has identified a novel polymorphism (a T/C variation at position 718) in the 3'UTR of the GPX4 gene. Both allelic variations of this single nucleotide polymorphism (SNP) are common in Caucasians within the UK. The functional significance of this SNP is being determined both in studies with cells transfected with reporter constructs and in a supplementation trials in which individuals are supplemented with 100ug/day sodium selenite for 6 weeks and then studied during the 'wash-out' period as their Se status returns to normal. In addition, disease association studies are being carried out with patients suffering from ulcerative colitis and colon cancer.

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HEPCIDIN DECREASES FERROPORTIN (FPN1) PROTEIN LEVELS AND NONHEME IRON RELEASE FROM J774 MOUSE MACROPHAGES AFTER ERYTHROPHAGOCYTOSIS.

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Hepcidin is a peptide hormone secreted by the liver in response to inflammation and iron loading. Recent data in HEK293 cells demonstrate that hepcidin binds to the iron-export protein, FPN1, and causes its degradation (Nemeth et al., 2004). Because 80% of iron in the circulation represents iron released from reticuloendothelial macrophages, fluctuations in macrophage FPN1 levels may affect serum iron concentrations. To model physiologic iron recycling by the macrophage, we examined the effect of hepcidin on FPN1 protein levels and 59Fe release in a mouse macrophage cell line after phagocytosis of 59Fe-labeled erythrocytes. After erythrophagocytosis, J774 cells were treated with vehicle or 700 nM hepcidin and harvested 3, 6, 12, and 22 h later. We found that hepcidin treatment markedly reduced FPN1 protein levels at all time points after erythrophagocytosis. The down-regulation of FPN1 was associated with a 50% reduction ($P < 0.001$) in nonheme 59Fe release 22 h after erythrophagocytosis, suggesting that FPN1 participates in nonheme 59Fe export after red cell ingestion. These in vitro data, if extrapolated to the in vivo situation, strengthen the associations between hepcidin, FPN1, and serum iron levels.

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GLUCOCORTICOID RECEPTOR ACTIVITY IS REGULATED BY METALLOTHIONEIN IN MOUSE FIBROBLASTS AND THYMOCYTES

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Metallothioneins (MTs) may regulate zinc availability to zinc-sensing and zinc-requiring cellular macromolecules, including transcription factors. We have reported that zinc-associated MT (ZnMT) in mammalian cells correlates with activity of the zinc-dependent glucocorticoid receptor (GR) induced by dexamethasone (*dex*). We report here that the capacity of GR to mediate transcription of an exogenous GR-responsive reporter construct, and endogenous GR-responsive genes (those encoding MT-1, IκBα, and GILZ) is impaired in MT-KO cells. GR-non-responsive GAPDH gene expression was unaffected. We also assessed the effect of MT loss on the capacity of primary mouse CD(+)CD8(+) thymocytes from MT-KO mice to apoptose in response to dex. Glucocorticoids induce apoptosis in CD4(+)CD8(+) thymocytes. We report that: a) MT-KO and MT-WT thymocytes from mice on zinc-deficient diets had reduced capacity to undergo dex-induced apoptosis, supporting the hypothesis that GR is regulated by zinc, and b) MT-KO mouse thymocytes had decreased capacity to undergo dex-induced apoptosis regardless of dietary zinc. These data support the concept that GR-regulated transcription of endogenous GR-responsive genes is regulated by MT-1 and/or MT-2, and the contention that there is a functional relationship between MT and GR-mediated physiological events.

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CYTOKINE EXPRESSION AND SELENIUM STATUS IN RESPONSE TO SELENIUM SUPPLEMENTATION IN ADULT SUBJECTS

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Previous data from this laboratory indicated that UK subjects consuming small dietary supplements of selenium produced variable increases in functional measurements of selenium status and an augmented cellular immune response (Broome et al. 2004). The aim of this study was to use existing samples from this previous study to assess variability in selenium-dependent responses in the immune system and to identify any markers of immune function that respond to dietary selenium intake. 60 lymphocyte samples taken at baseline and post-supplementation from 30 healthy adult subjects were utilised. Adult subjects had been allocated into 3 groups and had received 50 or 100 µg selenite or placebo daily for 15 weeks. Lymphocyte samples were activated with mitogen and cytokine responses were assessed using cDNA arrays. Data indicate that supplementation of adult subjects with selenium modified a small number of mitogen-stimulated changes in lymphocyte cytokine gene expression, but there was a substantial inter-individual variability in the magnitude of responses. The mean data from the cytokine arrays supports our previous findings that selenium supplementation induces small changes in a number of lymphocyte cytokine responses to stimulation. A number of transcripts for specific cytokine genes showed changes in response to selenium supplementation and/or were related to the size of the selenite exchangeable pool. The levels of these genes might be potential indicators of selenium-sensitive aspects of immune function.

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MUTATION ANALYSIS IN THE ATP7B GENE OF WILSON DISEASE PATIENTS AND CORRELATION WITH THEIR PHENOTYPES SUGGEST POTENTIAL ROLE OF OTHER MODIFIER LOCI IN THE DISEASE

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Wilson disease (WD) is an autosomal recessive disorder caused by mutations in a copper-transporting P-type ATPase gene (ATP7B) resulting in the accumulation of copper in the liver and the brain. WD mainly has hepatological, neurological or mixed manifestations. The objective of our study is to identify the prevalent mutations in the Indian population and to establish a genotype-phenotype correlation. So far WD patients from 80 unrelated families have been recruited in this study. Three dinucleotide repeat markers flanking WD locus and a few intragenic SNPs were used to determine the genotypes and construct the haplotypes of the patients. Mutation screening revealed four prevalent mutations that accounted for about 37% of all the mutations. Work is in progress to identify other major mutations. Interestingly, homozygotes for different mutations that would produce similar defective proteins showed significant disparity in terms of organ involvement and disease severity. Also in one family, the proband and a sib had remarkably different phenotypes in spite of sharing the same set of mutant chromosomes. These findings suggest the potential role of yet unidentified modifier loci for the observed phenotypic heterogeneity among the WD patients. We also observed neurological WD patients with little or no clinical evidence of hepatic involvement though reports in the literature suggest early hepatic involvement compared to neurological symptoms (El-Youssef. 2003).

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ELEMENT ANALYTICAL METHODS: USEFUL AIDES AND ESSENTIAL PARTNERS IN TRACE ELEMENT AND METALLOPROTEIN RESEARCH

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Studies on the distribution and metabolic fate of trace elements and on the relationships between imbalances in the element status and diseases require a large number of elemental analyses in tissues, diets or environmental materials. Various procedures for both single element and multi-element determination have therefore been developed which allow routine analyses to be carried out on a large scale. In addition, however, specific element analytical methods may also be applied in the forefront of trace element research, such as tracer techniques with radionuclides and enriched stable isotopes or scanning procedures for the determination of the trace element distribution in biological samples. Advanced analytical methods are of special importance in the identification of novel metalloproteins. As their properties are not yet known, first information on their existence in tissues or cells can only be obtained by combining methods of protein separation and characterization with suitable procedures for the determination and localization of metals and metalloids in the isolated proteins. In this overview the applications of various element analytical methods in trace element and metalloprotein research and their advantages and disadvantages are discussed with the help of some examples.

ISOTOPE DILUTION ICP-MS AND ICP-MS HYPHENATED TECHNIQUES AS AN ACCURATE AND SENSITIVE TOOL FOR TRACE ELEMENT AND METALLOPROTEIN ANALYSIS.

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Isotope dilution mass spectrometry (IDMS) is internationally accepted as a precise and accurate analytical method for the determination of trace elements in different matrices (Heumann 2004). By using spike solutions, which are enriched in stable isotopes of the elements to be determined, single-element and multi-element ICP-IDMS can be carried out with all different kinds of human and animal samples. For ICP-MS measurements the sample preparation can either be done by microwave assisted decomposition with oxidising chemicals like HNO_3/H_2O_2 or direct analysis can be carried out by laser ablation (LA-ICP-MS) after the isotope dilution step. Corresponding silicon and platinum trace determinations are presented. Coupling of ICP-MS with separation techniques like high performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) also allows to determine the chemical form of elements (elemental species) under certain conditions. GC/ICP-MS coupling is used to demonstrate the determination of siloxanes in human tissues from women with breast implants. Size exclusion(SEC)-HPLC and reversed phase(RP)-HPLC coupled with ICP-MS are applied to determine metalloproteins in healthy and cancerous thyroid samples and CE-coupling in combination with the isotope dilution technique is used for the characterisation and quantification of metalloprotein isoforms.

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USE OF MALTOL AND CLOSE ANALOGUES TO EITHER IMPROVE ABSORPTION OR SPEED EGRESS OF TRACE METAL IONS. LESSONS FROM CELLULAR, ANIMAL MODEL AND HUMAN STUDIES

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The importance of chelating agents as regulators of trace metal ion uptake, distribution and excretion is gaining increasing attention, as part of a greater appreciation of metal ions in medicine (Thompson and Orvig, 2003). Key molecular features of hydroxyl-pyrones and –pyridinones, of which maltol is a simple example, are a readily ionizable proton and a zwitterionic aromatic character, permitting formation of bidentate metal complexes with these ligands, that are thermodynamically stable, have a reasonable hydrophilic/lipophilic balance, and possess neutral charge. Our experiments with radiolabelled vanadium and gallium hydroxy-pyrone and –pyridinone complexes in rats, mice and rabbits have demonstrated the capacity of maltol and similar ligands to either enhance tissue uptake or speed body clearance of particular metal ions. In a Phase I clinical trial in humans, the relative bioavailability of vanadium (measured by ICP-MS), administered orally as bis(ethylmaltolato)oxovanadium(IV), was three times that from the corresponding inorganic oxovanadium(IV) compound, vanadyl sulphate. In cell studies, maltolato complexation has tended to enhance cell uptake of a variety of metal ions, both in our studies and those of others. Overall, these results point to a growing need to take into account ligand effects on trace and ultratrace metal bioavailability in estimating appropriate doses of metal-containing therapeutics, whether for supplement or medicinal use. Possible mechanisms of maltolato effects on the uptake and clearance of trace metal ions are presented.

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OBSERVATION OF STRUCTURAL CHANGE OF METALLOTHIONEIN ISOFORMS USING CAPILLARY ZONE ELECTROPHORESIS

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Capillary zone electrophoresis (CZE) is an effective separation method for the analysis of metallothionein (MT) isoforms. However, unfavorable adsorption to the inner wall of an uncoated capillary tube is a big problem. We made polybrene and polyacrylic acid double coated (PPA-coated) capillary for the separation of MTs. The effective characteristic of a PPA-coated capillary is that the inner wall is charged more strongly than an uncoated fused silica capillary. Therefore, positive, neutral, and negative charged substances can be simultaneously separated in a PPA-coated capillary. Cd-MT-1 and Cd-MT-2 were migrated in the negative zone using a PPA-coated capillary at pH 7.4. And the peaks were disappeared from the original positions after EDTA was added to Cd-MT solution. MT was identified in the neutral area, where the peak increased after EDTA addition, using a MALDI-TOF mass spectrometer. The original peaks of MTs also changed when several chemicals such as NO donor and hydrogen sulfide were added. It is concluded that a PPA-coated capillary is of benefit for the observation of the properties of MT isoforms owing to its ease of manufacture and its detecting abilities at a wide charge range.

A VALID HPLC METHOD FOR PHYTATE AND ITS APPLICATION TO ZINC DEFICIENCY DIAGNOSIS

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A validated HPLC method for the analysis of phytate is available. Ferric-sulfosalicylic acid forms a stable colored complex under acid conditions, releases the ferric ion to ligands such as phosphate and inositol phosphates. Conditions for these reactions are critical. Details of HPLC method are available from the authors. The reproducibility within labs., "paired t" for 64 pairs of data, had a probability > 0.05. Sample concentration of the 8 pairs across all laboratories (Mean ± SEM) was 1) 9.6 ± 0.44, 2) 5.4 ± 0.26, 3) 0.3 ± 1.20, 4) 5.7 ± 0.28, 5) 9.6 ± 0.38, 6) 12.7 ± 0.47, 7) 9.2 ± 0.32, and 8) 19.6 ± 0.63 respectively. Samples 3 and 14 content were below detection limit for 5 of 8 labs., otherwise excellent agreement among laboratories. COV across labs. were 10 to 20%. Method was used study of zinc deficiency in a sample of the Maya population in Guatemala. Indigenous food samples were purchased for analysis of phytate using the method. Zinc was analyzed by AA after microwave ashing. Growth rate in children, height and weight for age for Maya infants and children were studied for 3 years. Standards were the 50-percentile established by the NCHS, NIH. In conclusion, any phytate:zinc molar ratio greater than 10 for an individual or group of individuals constitutes zinc deficiency by definition.

SPECIATION OF SELENIUM IN YEAST AND GARLIC IN RELATION TO CANCER PREVENTION

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A range of research supplements containing selenised yeast were characterised by HPLC-ICP-MS and HPLC-ESI-MS following enzymatic degradation of selenium-containing peptides in the yeast. The results, which showed a varying inter-batch pattern of selenomethionine and many unknown constituents, raise the question of

the need for improved production quality assurance. The absorption rate of selenium from a yeast supplement was estimated in a human intervention study by the stable isotope approach using intrinsically 77Se-labelled yeast. The results, which were acquired for 76Se, 77Se and 80Se by an ICP-MS instrument equipped with a dynamic reaction cell (DRC) using human blood plasma, urine and faeces as samples, translated into an absorption rate and retention of selenium at 89 % and 74 %, respectively.

In contrast to yeast, plants from the Allium family biosynthesise a different pattern of selenium species, the predominant one being gamma-glutamyl-methylselenocysteine. Several detected minor selenium species included propyl-selenocysteine. In analogy with S-allyl-cysteine (allicin) being abundantly present in garlic, an attempt was made to find Se-allyl-selenocysteine. The expected enzymatic degradation of this selenium species was suppressed by inhibiting the alliniase enzyme in garlic by hydroxylamine during the extraction process. The results showed however, that Se-allyl-selenocysteine was absent in the extract. Selenium-enriched garlic has been shown higher cancer preventive efficacy in comparison with yeast, an effect, which might be associated with its high content of gamma-glutamyl-methylselenocysteine in garlic.

CO-EXISTING MICRONUTRIENT DEFICIENCIES IN DEVELOPING COUNTRIES: IMPLICATIONS FOR INTERVENTION PROGRAMMES.

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Co-existing micronutrient deficiencies in developing countries are gaining increasing recognition, prompted by the often disappointing responses observed with single micronutrient interventions. Multiple micronutrient deficiencies can limit the effectiveness of programmes aimed at single micronutrients. Examples include the failure of supplementation with: (a) iron to improve hemoglobin when deficiencies of vitamin A, riboflavin, vitamin B-12, and/or folate co-exist; (b) vitamin A to restore night vision when zinc deficiency co-exists; (c) iodine to improve thyroid function when deficiencies of iron or selenium co-exist. Consequently, strategies should focus on identifying and alleviating co-existing multi-micronutrient deficiencies. Results of multi-micronutrient efficacy trials have been mixed, however, attributed in part to differences in the age and nutritional status of study group at baseline, the duration and design of the study, and the dose, form, and combinations of multi-micronutrients used. Interactions between supplemental micronutrients and chelating substances in habitual diets may also play a role by compromising bioavailability and in some cases, producing negative side effects, especially in malnourished individuals. Clearly, innovative approaches are needed to minimize the risk of antagonistic interactions in multi-micronutrient interventions. Possible new strategies include using combinations of weekly and daily supplements, microencapsulated or protected fortificants, and multi-micronutrient fortified sprinkles and fat-based spreads. More sustainable strategies suitable for subsistence farming settings include dietary modification/diversification and biofortification, approaches that can increase intakes of readily available micronutrients for the entire household.

THYROID FUNCTION AND SELENIUM DEFICIENCY IN HUMANS: A REVIEW

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When iodine supply is normal, no clinical effects of selenium deficiency on physical growth or intellectual development are apparent an only minor biochemical changes in thyroid hormone concentrations are observed with selenium supplement (Calomme et al. 1995). Combined iodine- and selenium-deficiency in Central Africa is associated with endemic myxedematous cretinism. Thiocyanate overload, a well-known goitrogen in these areas, could precipitate the involution of thyroid gland (Contempré et al. 2004). In Tibet, the prevalence of endemic goiter in children is markedly lower than in endemic areas of Congo, despite lower urinary iodine concentrations. The staple food, barley, does not contain thiocyanate. Selenium deficiency is definitely more severe than in Central Africa. The clinical profile of cretinism (neurological form) is different from the one observed in central Africa (Moreno et al. 2003). Nutritional covariables like selenium deficiency and thiocyanate overload are likely the effect modifiers of the clinical profile associated with severe iodine deficiency.

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TOLERABLE UPPER INTAKE LEVELS OF TRACE ELEMENTS – RECENT ADVANCES

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Recently, science based risk assessment methods have been applied to the establishment of Tolerable Upper Intake Levels (UL) for micronutrients by expert panels, including the EU Scientific Committee on Food (http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html) and the European Food Safety Authority (http://www.efsa.eu.int/science/nda/nda_opinions/catindex_en.html), and the Food and Nutrition Board of US Institute of Medicine (http://www.iom.edu/project.asp?id=4574). This represents a significant advance in providing dietary reference standards for evaluating and managing the risk of over-consumption of these nutrients. UL are increasingly used in nutritional surveillance as studies on nutrient intakes in populations give greater attention to the possibility of over-consumption of micronutrients resulting from wider use of nutritional supplements and consumption of fortified foods. UL may also be used for setting maximum levels of nutrient addition to foods and supplements. These scientific assessments have revealed significant limitations in the data on dose response relationships for adverse health effects of trace elements in humans. In order to improve understanding of such effects research is needed to provide better knowledge of the physiological effects of trace elements at the molecular, cellular and whole body levels, and of kinetics of their absorption, metabolism and excretion at different dietary levels of intake.

MERCURY: AN ELEMENT OF MYSTERY

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Methyl mercury is one of the most insidious and toxic forms of this element. It is present in virtually all species of fish and marine mammals. The main if not sole source today is the bio-methylation of inorganic mercury present in aquatic sediments followed by bio-accumulated in aquatic food chains. Methyl mercury levels in edible tissues are usually at sub toxic levels but outbreaks of poisoning have occurred in areas of local pollution and perhaps insidious adverse effects have arisen due to excessive intake of marine or freshwater food. A remarkable property is the ease with which methyl mercury is absorbed from food, circulated to body tissues, and transported into cells. It turns out that methyl mercury forms a complex with the amino acid, cysteine, to gain entrance into cells on the large neutral amino acid carrier. At first glance it would seem difficult to explain the evolution of this rapid entry of this highly toxic molecule into cells on a universal carrier. This presentation will attempt to make the case that entry into the cell is, in fact, a protective mechanism at least at low ambient levels of methyl mercury. It is proposed that the sensitive targets are on the cell surface whereas the cell interior has adequate protective mechanisms. Toxic action on the cell surface and protection inside the cell may explain some apparent discrepancies in the outcomes of recent epidemiological studies of populations consuming methyl mercury in marine food.

THE ASSOCIATION BETWEEN BASELINE SELENIUM, ALPHA AND GAMMA TOCOPHEROL, AND THE EFFECT OF SELENIUM SUPPLEMENTATION ON CANCER INCIDENCE

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Selenium supplementation has been shown to be associated with a reduction in cancer incidence, particularly prostate cancer (PCa). It has been suggested that the efficacy of selenium can be affected by interactions with other nutrients, such as forms of vitamin E. The Nutritional Prevention of Cancer (NPC) trial, the largest clinical trial to date to have shown the efficacy of selenium supplementation, randomized 1312 subjects to either 200 mcg/day of selenized yeast or a matched yeast placebo. This report describes the extent to which baseline plasma levels of alpha-tocopherol (AT) (n = 1026), gamma-tocopherol (GT) (n = 1004), and selenium (Se) (n = 1250) modulate the effect of selenium supplementation on total cancer and PCa incidence. Se supplementation showed significant reductions in risk of total cancer [Hazard ratio (HR) = .62,95% confidence interval (CI) = .41-.93] and PCa (HR=.31,95%CI=.12-.80) among those with a baseline plasma AT level below the median. Conversely, at baseline plasma GT levels above the median (*high*), Se supplementation showed the greatest reduction in risk for total cancer (HR = .63, 95%CI = .41-.96) and PCa (HR = .31,95% CI = .13-.73) incidence, compared to baseline plasma GT levels below the median (*low*). When considering the effect of baseline plasma Se and AT, a significant reduction in total cancer risk occurred with Se supplementation when AT was high and baseline Se was low (HR = .44, 95% CI = .21-.92). Again, high baseline GT and low baseline Se produced significant reductions in total cancer with supplementation (HR = .38, 95% CI = .18-.83). These data suggest that baseline plasma concentrations of AT, GT, and Se may influence the efficacy of Se supplementation and that GT and Se may play a more important role in cancer prevention than has been previously recognized.

INCREASED THYROID VOLUME IN CHILDREN WITH HIGH DIETARY IODINE INTAKE

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There are few data on the adverse effects of chronic exposure to high iodine intakes, particularly in children. Our objective was to determine if high dietary intakes of iodine increase thyroid volume (Tvol) and/or risk for goiter in children. In an international sample of 6-12 y-old children (n = 3319) from 5 continents with iodine intakes ranging from adequate to excessive, Tvol was measured by ultrasound and urinary iodine concentration (UI) determined. Regressions were done on Tvol and goiter including as covariates age, body-surface area, gender, and UI concentration. The median UI ranged from 115 μg/L in central Switzerland to 728 μg/L in coastal Hokkaido, Japan. In the entire sample, 31% of children had UI > 300 μg/L and 11% had UI > 500 μg/L; in coastal Hokkaido, 59% had UI > 500 μg/L, and 39% had UI > 1000 μg/L. In coastal Hokkaido, the mean age- and BSA-adjusted Tvol was ≈ 2-fold greater than the mean Tvol from the other sites combined ($p < 0.0001$) and there was a positive correlation between log(UI) and log(Tvol) ($r = 0.24$; $p < 0.0001$). In the combined sample, after adjusting for age, sex and body surface area, log(Tvol) began to increase at log(UI) > 2.7, which, transformed back to the linear scale, corresponded to a UI concentration of≈500 μg/L. In conclusion, chronic iodine intakes ≈ 2 fold higher than recommended—indicated by UIs in the range of 300-500 μg/L – do not increase Tvol in children. However, UIs ≥ 500 μg/L are associated with increasing Tvol, reflecting the adverse effects of chronic iodine excess.

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EFFECT OF ZINC SUPPLEMENTATION ON THE IMMUNE FUNCTION OF HEALTHY OLDER INDIVIDUALS AGED 55-70 YEARS: THE ZENITH STUDY

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Zinc plays a vital role in the immune system and approximately 50% of adults aged 50 -70 years may have sub-optimal zinc status [1]. The current study aimed to determine if 15 mg or 30 mg of zinc (as zinc gluconate) per day for six months affected the immune function of 93 individuals (45 men & 48 women) aged 55-70 years. Multiple flow cytometric methods were used to assess immune function. At baseline, serum and erythrocyte zinc concentrations, and dietary zinc intake were 13.0 (± 1.4) μM/l and 22.2 (± 4.8) μM/l, 9.9 (± 4.0) mg/d, respectively. Treatment effect was determined using general linear mixed effects model. A significant time x treatment interaction was observed for monocyte count ($p = 0.026$), total naive T-cells ($p = 0.036$), and % T-cell production of INF-α (p 0.034). Both the % expression and absolute count of B-cells showed a significant decline in individuals receiving 30 mg Zn/d compared to 15 mg Zn/d at month 3 only. TNF-α production by T-cells (ABC) showed a significant increase in both treatment groups compared to placebo group at month 3 only. These findings indicate that zinc intakes of up to 40 mg zinc/d in healthy individuals do not appear to affect immune function in apparently healthy 55-70 year olds.

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ULTRA-TRACE ELEMENTS (UTES) MAY NEED TO BE REGULATED IN INFANT FORMULA PREPARATIONS

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The long-term goal of our work (Collaborator-Curtis Hunt, USDA, ARS, Grand Forks, ND) is to assess UTEs in infant formulas (F) available in North America. UTE content of F is not regulated either by law or by manufacturing practice. UTEs appear solely as contaminants depending on the source of protein. We have shown (Friel, 1999; Hunt, 2004) that both boron (B) and molybdenum (Mo) appear to be under homeostatic control in human milk (HM). As a first step to a North American survey, we analyzed data from our previous work. Results for 5 (Mo), and 2 (B) different F compared to HM for Mo; (X (range): F, 23 (6–46) ug/L vs HM, 5 (2-23) ug/L; B: F, 120 (90-150) ug/L vs HM, 30 (20-60) ug/L indicate higher quantities of both UTEs in F. At present, we are collecting representative F and analyzing for UTEs to confirm these findings. Mo and B may be essential and our data suggest there is merit in regulating F content of UTEs.

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SELENOPROTEOME

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Selenocysteine (Sec) is a rare amino acid which occurs in proteins in major domains of life. It is encoded by UGA, which also serves as the signal for termination of translation. Consequently, most selenoprotein genes are misannotated. Information on full sets of selenoproteins (selenoproteomes) is essential for understanding the biology of the trace element, selenium. We identified selenoprotein genes in completely sequenced genomes by methods that rely on identification of selenocysteine insertion RNA structures, the coding potential of UGA codons, and the presence of cysteine-containing homologs. The human selenoproteome consists of 25 selenoproteins, whereas the size of other selenoproteomes varies from 1 to 32 selenoproteins. We are functionally characterizing several selenoproteins and linking their functions to biomedical effects of dietary selenium, including roles in cancer prevention, aging and male reproduction. Most selenoproteins were found to be oxidoreductases containing the active site selenocysteine. We will also discuss other applications of selenoproteomes, including characterization of translational mechanisms and identification of redox cysteines in protein sequences on a genome wide scale.

SEARCHING FOR BIOMARKERS OF EARLY EFFECTS OF COPPER EXPOSURE IN HUMANS

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Uptake, efflux, storage and utilization of Cu preventing adverse effects due to Cu excess is tightly regulated in man, but the upper and lower limits of homeostatic regulation are unclear because there are no biomarkers able to detect early and less intense Cu effects. We have conducted a series of studies assessing potential indicators of early changes of Cu status in humans in response to mild-moderate copper excess. With the hypothesis that "healthy" individuals would respond to Cu exposure in accordance with their position in the population serum ceruloplasmin (sCp) distribution curve, we assessed the effect of the Upper Level of dietary recommendations in a prospective controlled trial in "healthy" adults. Results showed that after 2mo liver aminotranferases activities remained below the cut off used to diagnose clinical liver dysfunction, but group means significantly increased in both low and high sCp groups. These differences disappeared after 12mo finishing the Cu load. Glutathion in MNC also increased in both sCp group ($p = 0.01$). SOD and CCS mRNA expression in MNC (done in collaboration with Prof. J Prohaska) significantly changed their expression, and hCTR1 but not DMT1 mRNA expression in monocytes significantly increased after the 2mo load. Principal Component Analysis and Linear Discriminant Analysis revealed that the sCp groups assessed behaved differently and responses detected depended on Cp values and on sex. sCp was further assessed as indicator of copper homeostasis in 68 "healthy" adult men that received 8 mg Cu/day (as $CuSO_4$,) for 6 months, in a blind fashion. Controls received a placebo of similar appearance. Results showed that the Exp and Control groups did not differ in their responses to Cu supplement but the subgroups representing the lower and higher terciles of the sCp distribution in the group responded differently; in the lower Cp group sCu decreased after supplementation while the high sCp group sCu remained unchanged (ANOVA repeated measurements, $p < 0.02$). The non-Cp Cu pool decreased in the low sCp group after 6mo while it increased in the high sCp group (ANOVA repeated measurements, $p < 0.001$). We would like to speculate that the low sCp phenotype may be due to a specific genetic polymorphism that affects whole body copper metabolism.

EFFECTS OF ELEVATED DIETARY ZINC ON NOVEL BIOMARKERS OF COPPER STATUS AND REGULATION OF INTESTINAL ZINC TRANSPORTERS.

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Intakes of high dietary zinc cause poor copper status in experimental animals and humans, however, the molecular mechanism by which zinc interferes with copper absorption is not known. Recently, we reported that the copper chaperone for Cu/Zn superoxide dismutase (CCS) is up regulated in copper-deficient rats. In this study we examined the suitability of CCS as a biomarker of copper status in rats fed varying intakes of zinc. Weanling rats were fed one of four diets containing normal (30) or elevated levels (60, 120 or 240) mg zinc/kg diet and containing normal copper (6 mg/kg) for 5 weeks. Liver zinc levels were increased in rats fed the high zinc diets (Zn-60, Zn-120, Zn-240) compared to rats fed normal zinc (Zn-30), confirming our experimental model. Liver, kidney and plasma copper levels were decreased, while liver, WBC and RBC CCS expression was increased in rats fed the Zn-60 and/or Zn-120 diet. CCS protein was determined to be the most sensitive biomarker of poor copper status. Surprisingly, rats fed the highest level of zinc (Zn-240) showed overall better copper status than rats fed the Zn-60 or Zn-120 diet. Interestingly, duodenal metallothionein-1 expression was markedly increased in rats fed the Zn-240 diet. Together, these data show that CCS protein is a good indicator of poor copper status induced by high dietary zinc and suggest that the activity of key intestinal zinc transporters also respond to high intakes of zinc and may modulate the effects of high zinc on copper status.

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BUCCAL CELLS AS A SOURCE OF MARKERS FOR ANALYSIS OF MICRONUTRIENT STATUS

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Inadequate intake of micronutrients is a major public health problem. Zinc deficiency has an estimated contribution to global burden of disease of 1.9% in terms of disability-adjusted life years. Despite the significance of zinc in human nutrition, there currently are no reliable indicators of body zinc status. Plasma zinc levels are commonly used as an indicator of zinc status, but may fluctuate according to variations in physiological states and therefore do not provide an accurate estimation of body zinc status. We have developed a novel technique for obtaining buccal cell mRNA and protein from mouthwash samples for molecular analysis (Michalczyk et al, 2004). We have used this method to detect zinc transporter molecules that are members of the recently identified SLC30 and SLC39 families. Members of this family are postulated to have a major function in body zinc homeostasis and may provide indicators of body zinc status. Using SYBR green real time PCR, we measured the relative mRNA levels of SLC30 and SLC39 zinc transporters. Our results indicate that mRNA levels of several members of the SLC30 family are reduced in individuals who are known to have inherited forms of zinc deficiency. Analysis of cellular markers from mouthwash cells is a non-invasive method that has the potential to be a rapid diagnostic tool for determining micronutrient status.

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CERULOPLASMIN. AN INDICATOR OF COPPER STATUS IN "NORMAL" POPULATION?

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Serum Ceruloplasmin (sCp) is a well accepted indicator of copper transport in patients with copper related diseases. Our previous studies on copper homeostasis showed that "healthy" adults that are at the lowest and highest decile of sCp behave differently when challenged by a 10 mg/d copper supplement for 2 months. This suggests that sCp may serve to discriminate special population subgroups in terms of sensitivity to high copper exposure. With the objective of testing this hypothesis 68 apparently healthy adults received in a blind fashion a daily pill for 6 months containing either 8 mg Cu/day (as CuSO4, Exp) or a placebo of similar appearance (Cont). Results showed that general characteristics (body weight, hemogram, glycemia, cholesterol profile), sCu, sFe, sZn, eSOD, glutathione (PMNC), liver aminotranferases and LDH, glycemia, total-, LDH- and HDL- cholesterol did not differ in Exp from Cont before, after 3mo and 6mo Cu supplementation. However subgroups representing the lower and higher terciles of the sCp distribution in the group showed a significantly different response to Cu supplement: in the lower Cp group sCu decreased after supplementation while in the high sCp group sCu showed no change. The non-Cp Cu pool decreased in the low sCp group after 6mo, but it increased in the high sCp group. These results support the notion that basal sCp in "healthy" subjects is an indicator of copper homeostasis. We would like to speculate that the low sCp phenotype may be due to a specific genetic polymorphism that affects whole body copper metabolism.

INFLUENCE OF THE INTERACTIONS BETWEEN DIETARY ZINC INTAKES AND CHRONIC CADMIUM EXPOSURE ON THE EXPRESSION OF METALLOTHIONEIN, NRAMP2 AND ZNT-1 EXPRESSION IN RATS

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The objective of this study was to investigate whether transporters involved in zinc transport played a role in cadmium-induced tissue zinc redistribution in rats. Twenty-one-day old SD rats were randomly assigned to one of the four dietary treatment groups: low-zinc (6 mg/kg diet), adequate-zinc (30 mg/kg diet) pair-fed control and ad libitum control, and supplemented-zinc (150 mg/kg diet) group. After 4 weeks, rats were exposed to cadmium at 0, 5, or 50 mg/kg diet for 5 weeks in a 3 × 3 factorial design (n = 6). Cadmium exposure had no effect on body weight and feed intake regardless of dietary zinc intake level. Cadmium exposure at 50 mg/kg diet increased liver zinc concentration in adequate- and supplemeted-zinc rats and kidney zinc concentration in adequate-zinc rats and reduced tibia zinc concentration in low- and adequate-zinc rats. Cadmium exposure at 5 g/kg diet reduced tibia zinc concentration in adequate-zinc rats. Cadmium exposure had no effect on duodenum zinc concentration regardless of zinc intake and cadmium exposure levels. Cadmium exposure (50 mg/kg diet) increased hepatic Nramp2 mRNA level in low-zinc rats, but not in adequate- and supplemented-zinc rats. Cadmium exposure (50 mg/kg diet) had no effect on mRNA level of metallothionein and ZnT-1 in duodenum, kidney and liver regardless of zinc intake.

Overall, the data showed that chronic cadmium exposure altered tissue zinc distribution, but this altered tissue zinc distribution appeared to be indipendent to the expression of metallothionein, Nramp2 and ZnT-1 in rats.

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ZINC AND PSYCHONEUROIMMUNOLOGY

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The field of psychoneuroimmunology was first described around 20 years ago and since then there has been an accumulation of experimental and clinical evidence supporting the existence of close and bidirectional communication between the immune system and the central nervous and neuroendocrine systems. Cognitive decline is an occurrence commonly associated with ageing, with many older people experiencing diminished learning and memory capacities. Similarly immune function is known to decline with age (immunosenescence) contributing to increased incidence of morbidity and mortality and it has been proposed that improved immune function may be closely related to the amelioration of age-associated cognitive deterioration. Optimal nutrition may be of critical importance in the maintenance of the immune system and prevention of cognitive decline. One nutrient that may have a significant role in linking immune function and cognitive function is zinc. Zinc has well-established functions in many aspects of the immune system: being vital in immune cell development and functioning; having a role in acquired immunity and being an essential co-factor for the thymic hormone, thymulin. It is also found at high concentrations in parts of the brain and its deficiency is associated with neuropsychological impairment as well as dementia. Older people may also be a potentially vulnerable sub-population owing to their lower dietary zinc intakes. It has been hypothesised that zinc dependent alterations in immune function may have a significant role in cognitive decline in ageing. Optimising zinc status in this vulnerable group may offer protection from cognitive decline, neurological conditions or neuro-psychiatric conditions such as Alzheimers disease or depression. The potential of dietary nutrients arresting declining mental health in older people is of major public health significance given the anticipated changes in population demographics in the years ahead.

RODENT MODELS DEMONSTRATE BIOCHEMICAL AND BEHAVIORAL CONSEQUENCES OF MATERNAL COPPER DEFICIENCY

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Neonatal ataxia in lambs, infant death in Menkes disease, and gross brain abnormalities in laboratory copper-deficient guinea pigs stimulated studies to identify copper-dependent neurochemical mechanisms. Offspring of copper-deficient rat dams exhibit significant decreases in brain copper and iron and reductions in the activity and levels of cuproenzymes CCO, DBM, PAM, and SOD. Six months of copper repletion failed to restore metals to control levels and resulted in rats with abnormal sensory motor function including impaired auditory star! tle, exaggerated foot splay, and poor performance in accelerating rotorod tests. Cerebellar development is currently being monitored to test specific hypotheses related to altered brain iron and cuproenzymes by evaluating energy metabolism, norepinephrine deficiency, neuropeptide amidation, and antioxidant status. Studies with several wild-type murine strains and mutants have confirmed and extended rat work. Mice are more sensitive to perinatal copper deprivation than rats. One notable difference is that mice do not have altered brain iron. Cuproenzyme function is also altered in brains of copper deficient mouse neonates. Though dietary copper deficiency is rare in adult humans, based on studies in rodents it is possible that current recommendations for intake during pregnancy and lactation may be too low. It seems clear that both diet and genotype impact the phenotypic expression of copper status.

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ELEMENT STATUS OF HIGH SCHOOL STUDENTS FROM SOUTH URAL

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Hair samples of 199 relatively healthy students (46 males, 153 females) aged 18-22 years were investigated. Concentration of the following 25 chemical elements in the samples was determined: K, Na, Ca, Mg, P, Co, Cr, Cu, Fe, Mn, Zn, Se, As, I, Li, Sn, V, Si, Ti, Ni, Al, Cd, Pb, Hg, Sr. Analytical determination has been carried out using ICP-AES and ICP-MS methods. It was found, that the lowered contents of Co, P, and I are characteristic for the majority of the students. In spite of the fact that the average level of the I contents in hair is within the limits of recommended meanings, 62.8% of the surveyed girls and 62.1% of the young men suffer from the lack of I, that is characteristic feature of Orenburg area. The young men more often have lower Co (89.6%), P (48.3%), Cu (48.5%), Se (48.3%) and higher Fe (51.7%) hair content. The Na, Zn deficiencies and K, Cr surpluses are distributed to lesser degree among young men. Among the girls more often there are deficiencies of Co (63.6%), Zn (52.5%), P (51.9%), Cr (50.6%) and K (41.0%) and surpluses of Mg (64.7%) and Ca (57.1%). The Cu, Se deficiencies and Fe, Na surpluses are distributed to lesser degree among girls. 66.7% of girls and 72.4% of young men have the increased Mg hair content. The estimated Mg, Fe excesses are due to enlarged levels of these elements in drinking water and local foodstuffs. Analysis of individual diets shows that 100% of the tested students have deficiency of Se consumed with food. The overwhelming majority of the students consume F (89.9%), Zn (84.9%) and Cr (54.8%) insufficiently. Analysis of an actual nutrition shows the characteristic Ca deficiency in 96% of the respondents, their consumption of Ca makes only 46% from an adequate consumption level. Iodine deficiency is a characteristic feature of 94.5% surveyed and average iodine content in diets is approximately 3 times less than RDA. 87% of respondents receive with food only 68% of the necessary amount of zinc. Thus, the received data testify, that in this imbalance the deficiencies of such essential elements as K, Co, Se, I, P, Cu and surplus of Mg, Ca, Mn and Fe prevail. The carried out researches allow developing the recommendations aimed at normalization of the element status of the Orenburg area students with the help of nutrition correction and consumption of mineral complexes. The Mg, Fe, Mn excesses, Ca/P imbalance, Se, I deficiencies in students are probably due to peculiarities of elemental content of local drinking water (elevated Mg, Fe, Mn levels) and foods (elevated Mg, Fe and lowered I, Se), i.e. geochemical factors and technogenic pollution (metallurgy, mining, oil industry).

EFFECT OF COPPER STATUS, SUPPLEMENTATION, AND SOURCE ON PITUITARY RESPONSIVENESS TO EXOGENOUS GONADOTROPIN RELEASING HORMONE IN OVARIECTOMIZED BEEF COWS

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The effect of copper (Cu) status, supplementation, and source on pituitary responsiveness to exogenous gonadotropin releasing hormone (GnRH) was evaluated using nine ovariectomized Angus cows. Cows were considered Cu deficient based on liver Cu concentrations (< 30 mg Cu/kg DM) after receiving a low Cu forage-based ration supplemented with 5 mg molybdenum/kg diet DM and 0.3% sulfur for 216 d. Copper-deficient cows were stratified based on age, body weight, body condition score, and liver Cu concentration and randomly assigned to repletion phase treatments. Treatments included: 1) control (no supplemental Cu), 2) organic (ORG; 10 mg Cu/kg DM), and 3) inorganic (ING; 10 mg Cu/kg DM; $CuSO_4$). During the 159-d repletion phase all cows received exogenous progesterone. By d 77 of the repletion phase, supplemented cows were considered adequate in Cu. Liver Cu concentrations were greater in supplemented vs. non-supplemented control cows on d 77 ($P < 0.05$) and throughout ($P < 0.01$) the repletion phase, while liver Cu concentrations were not different between ORG and ING cows. On d 99, GnRH was administered at low (0, 3, and 9 μg) and high doses (0, 27, and 81 μg) every fifth day, and blood samples were collected every 15 min for 1 h pre-, and 4 h post-, GnRH administration and analyzed for luteinizing hormone (LH) concentration. Copper status, supplementation, and source did not affect GnRH-induced LH secretion or pituitary LH concentration in ovariectomized progesterone-supplemented cows in this experiment, indicating that Cu status may not affect pituitary LH production or release in beef cows.

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AN ASSESSMENT OF ZINC ADEQUACY IN THE UK POPULATION USING MEASURED ZINC INTAKE AND ADEQUACY THRESHOLDS DERIVED FROM THEORETICAL MODEL OF REQUIREMENTS

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Inadequate dietary zinc intake is one of the causes of zinc deficiency and assessment of the adequacy of zinc intake can be a method for assessing zinc status in a population.

Analysis of the data from National Diet and Nutrition Surveys (NDNS) using the UK Reference Nutrient Intake (RNI) and Lower Reference Nutrient Intake (LRNI) as cut-off points classified children, adolescent and the elderly as groups vulnerable to zinc inadequacy in the UK (Amirabdollahian & Ash, 2004). International Zinc Nutrition Consultative Group (IZiNCG) has proposed new cut-off points giving Estimated Average Requirement (EAR) values (Brown et al. 2004). In general IZiNCG EARs are lower than UK EARs in childhood and early adolescence but substantially higher for the adult population. A Reanalysis of the UK NDNS data, using the IZiNCG EARs shows few children (5.6%, NDNS 1992-93) and 56.5% of young people (NDNS 1997), would be classified with intake less than EAR. However, in adults (NDNS 1986-87), 59.3% (consisting of 43.6% males and 15.7% females) would be classified as less than EAR. Using these criteria, even higher numbers of the elderly would be categorised with intakes less than EAR; 82% (consisting of 46.5% males and 35.6% females) (NDNS, 1994-95). Therefore, both cut-off points suggest a problem with the zinc inadequacy in the UK elderly population. This analysis highlights the difficulties in defining the zinc adequacy of the diet and the use of this as a proxy measure of zinc status. There needs to be more research to reconcile the UK Daily Reference Values (DRVs) and IZiNCG proposed reference values.

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MATERNAL IRON DEFICIENCY IDENTIFIES CRITICAL WINDOWS IN CARDIOVASCULAR DEVELOPMENT IN THE RAT

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Reduced fetal growth is linked with development of disease in later life. When the fetus is most sensitive to the maternal environment, however, is not well identified. Here, we use our model of Fe deficiency, which causes high blood pressure in the offspring, to identify critical windows and to test whether they are different for different outcomes. Female Rowett hooded Lister rats were fed either control or Fe-deficient diet for four weeks before being mated. Dams were killed on gestation day 10.5 and dissected embryos were cultured in either control or Fe-deficient male rat serum. After 48 h, the morphology of the embryos and their yolk sacs were scored. Growth was impaired under all deficient conditions compared to the controls. Significantly, culturing Fe-deficient embryos in control serum could not reverse these effects. The heart was enlarged compared to the controls. The yolk sac vasculature of embryos cultured in Fe-deficient serum had a higher incidence of blood islands, and a thinner and less branched network than controls. Culturing embryos of Fe-deficient dams in control serum reversed these changes, demonstrating that day 10 to 12 of gestation are critical in the response of the circulatory system to maternal Fe deficiency. We conclude that there are critical periods during development when supplementation may be more beneficial than others.

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SELENIUM SUPPLEMENTATION OF CATTLE PROVIDED DIETS CONTAINING SUGARCANE MOLASSES

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The S x Cu antagonism is an important consideration in ruminant nutrition. Sugarcane molasses (SCM) is a widely used animal feed byproduct, but is high in S and will impact Cu status of cattle (Arthington & Pate, 2002). Dietary S may also antagonize Se; therefore, two 90-d studies were conducted, using forage-fed, yearling steers (n = 24/study) to investigate the impact of SCM supplementation (≈ 0.5% body weight, dry matter basis) on measures of Se status. In study 1, steers were assigned supplements providing equivalent amounts of energy and protein from two sources (SCM and corn). Supplemental Se was provided (2.5 mg/d; Na selenite) to both treatments. After 90 d of supplementation, steers provided corn diets had greater liver Se concentrations and plasma glutathione peroxidase (GPX) activity compared to steers supplemented with SCM. In study 2, sources of Se, fed within SCM supplements, were compared. Selenium sources (2.5 mg/d) included, 1) Na selenite, 2) Se-yeast (Sel-Plex®, Alltech, Nicholasville, KY), or 3) no Se (control). Cattle provided supplemental Se, irrespective of source, had greater liver and plasma Se concentrations and greater plasma GPX activity compared to control on d 30, 60, and 90. Measures of Se status did not differ among steers supplemented with Na selenite and Se-yeast. These data suggest that dietary S, derived from SCM, will antagonize Se in cattle. Selenium status of cattle consuming SCM is similar when provided 2.5 mg of supplemental Se/d from Na selenite or Se-yeast sources.

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INFLUENCE OF DIFFERENT DIETARY COMPONENTS ON POTENTIAL IRON BIOAVAILABILITY

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Iron fortification of wheat flour has been mandatory in Argentina since 2003. The aim of this study was to assess the influence of different dietary components on potential iron bioavailability in bread made with fortified flour. Samples subjected to analysis comprised mixtures of bread made with ferrous sulphate fortified flour with: 1) individual dietary components, 2) usually consumed beverages, and 3) whole infant diets made up with those ingredients and beverages. The individual ingredients were mashes of pumpkin, potato, wheat grits, apple and banana; the beverages were water, iron fortified milk, "mate" infusion and a 50:50 mixture of milk with a "mate" infusion. Potential iron bioavailability was assessed using an in vitro modified method, which measures iron dialyzability (FeD%) under controlled pH conditions, after a digestion simulating physiological processes. Bread FeD% (10.7) was not modified by pumpkin (12.5) or potato mashes (9.7); diminished significantly with apple (7.6), milk + "mate" (4.8) and "mate" infusion (5.1) and even more with wheat grits (3.0), banana (2.1) and milk (1.5). Bread FeD% in the whole diet together with water as beverage (14.0) diminished significantly when the beverages were either milk + "mate" (4.5) or "mate" infusion (5.3). These results show the effect of components of habitual infant diets on fortification iron potential bioavailability.

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ELEMENT CONCENTRATIONS IN BREAST AND POWDERED MILK SAMPLES

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Breast milk samples were collected from 2434 lactating women between 3 and 481 days after delivery. The samples were chosen from all areas of Japan. The samples were classified according to the elapsing days after delivery, and into winter and summer. Twenty-one brands of powdered milk samples were bought at general markets in Tokyo. Samples were classified for regular infants (n = 6), for follow-up after 9 months of birth (n = 5), for special treatment formula for milk allergy, lactose intolerance, high blood pressure (n = 8), for low body weights (n = 1) and for pregnant or lactating mothers (n = 1). Various element concentrations were determined by microwave induced plasma mass spectrometer or atomic absorption spectrometer. In the breast milk samples zinc concentrations were decreasing with elapse of time from over 5 to under 1 mg/L and those in summer were higher than in winter. Sodium concentrations were decreased from 3 days till 2 months from 350 to 150 mg/L and then reached stable levels. Time dependent change of potassium concentrations were similar to sodium; they decreased from 600 to 400 mg/L and then kept stable levels. Remarkable changes of selenium concentrations were not observed, 0.025 to 0.035 mg/L, but the summer samples were higher than winter samples during the first month. The other elements did not show remarkable seasonal variation. In the powdered milk samples noticeable points were very low copper and zinc concentrations in follow-up milk samples. These 2 elements have to be added by law to regular infant formula but not in follow-up milk powders in Japan. Calcium and phosphorus concentrations in mothers' milk were about twice that of the other powdered milk. There was a little difference in each brand.

THE EFFECTS OF MATERNAL ORGANIC IRON SUPPLEMENTATION ON PIGLET PERFORMANCE

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The piglet is born with low iron reserves and needs supplemental iron after birth to prevent anaemia. Uteroferrin, an iron-binding protein, is the major mechanism by which the transfer of iron from the sow to the developing foetus occurs. This is relatively unresponsive to inorganic iron, but responds to organic iron. A series of 8 commercial trials studied the effect of supplementing sow diets prior to parturition and throughout lactation with 90 mg Fe from organic iron (Bioplex Iron, Alltech Inc) on piglet performance. There was no difference in piglet birth weight, but weaning weight was increased from 6.17 (± 0.9) to 6.48 (± 0.9) kg. Mortality during lactation was reduced from 10.8 to 6.8%. A reduction in the proportion of small piglets at weaning from 13.3 to 8.1% and an increase in the proportion of larger piglets from 45.2 to 55.3% was also observed. In one of the trials organic iron increased the serum iron status of both the sow (2.46 to 2.97 mg%) and the piglets (1.44 to 2.63 mg%). These results suggest that the increased iron status of the new-born piglet, possibly through an increased transfer of uteroferrin, produces a stronger, more viable and active piglet at birth. Colostrum and milk intake is increased, mortality reduced and growth rate improved. This is of lasting benefit to subsequent growth and development of the pig.

ZINC CONCENTRATION ON PLASMA, URINE AND DIET OF INDIVIDUAL UNDERGOING A GASTROPLASTY WITH ROUX-EN-Y BYPASS

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Due to zinc importance on several biological processes, on surgical procedure and patients' convalescence, the study aim was to assess the mineral nutritional status in the surgical preoperative to observe

the influence of zinc in the subject's health. Twenty three patients (20 females and 3 males) were studied before Capella's surgery with Roux-en-Y bypass. Blood sample and 24 h urine were collected and three day food record (one of which had to be a weekend day) was employed to assess nutritional intake. The zinc concentration on the sample was analyzed by flameless atomic absorption spectrophotometry. Dietary analysis of the food records were performed using the software program Virtual Nutri (School of Public Health, Brazil). Mean zinc plasma concentration was 66.6 µg/dL (74% < 70µg/dL and 26% between 70 – 110 µg/dL) and mean urinary excretion was 842.73 µg/24 h (73% > 600µg/24h and 27% between 300 – 600 µg/24 h). Mean zinc intake was 17.71 mg for males (33% <EAR and 77% ≥ EAR) and 8.20 mg for females (44% < EAR and 56 ≥ EAR). This study suggests that plasma zinc concentration on the studied patients was lower than reference ranges while the urinary excretion was higher showing altered mineral distribution on the body, according to other studies in obese individuals. Nevertheless, zinc intake for both males and females was adequate to reference values.

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THE ROLE OF THE SECIS ELEMENT IN INCORPORATION OF SELENIUM INTO THIOREDOXIN REDUCTASE ISOFORMS

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Selenium is incorporated into mammalian selenoproteins by the re-coding of a UGA codon in the open reading frame. This cannot occur without the presence of the selenocysteine incorporation sequence (SECIS) element present in the 3'untranslated region (3'UTR). Some selenoproteins are more sensitive than others to decreased selenium supply; for experiments in rats cytosolic thioredoxin reductase (TR1) was more sensitive to selenium status than mitochondrial thioredoxin reductase (TR2). In order to determine if the SECIS element was critical in this response we generated constructs incorporating a UGA codon upstream and either the TR1 or TR2 3'UTR downstream of the luciferase gene. These constructs were transfected into Se-supplemented and Se-depleted rat liver and kidney cells (H4 and NRK-52E) and the luciferase activity measured. Cells transfected with the construct incorporating the TR1 3'UTR maintained a constant level of luciferase expression regardless of Se status. The Se-deficient cells transfected with the TR2 3'UTR containing construct demonstrated a small (but significant) loss of luciferase activity compared to the Se-replete cells. Thus it appears the TR2 SECIS element is largely responsible for expression of TR2 protein however it may not be the sole source of regulation for TR1.

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INTERRELATIONSHIPS BETWEEN BLOOD, ERYTHROCYTE AND DIETARY ZN LEVELS, IN RATS

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Blood Zn levels relationship with Zn erythrocyte levels was evaluated in female Wistar rats (280-350 g) fed during pregnancy and lactation with a diet (group AIN) prepared according to the American Institute of Nutrition (AIN-93) recommendations (casein: 20.0 g/100g; Zn: 35 µg/g). A second group, fed the usual commercial diet (CG), (protein: 26 g/100g; Zn: 116 µg/g) was run simultaneously. Maternal blood samples were drawn from the tail before mating (To), at delivery (Td) and at weaning (Tw). Zn and Haemoglobin (Hb) were assessed in whole blood (WB) and in washed and haemolysed erythrocytes (RBC). Zn was determined by Atomic Absorption Spectrometry. The results (mean ± SEM) were: WBZn (µg/dL): To: 470 ± 23; CG: Td: 553 ± 36; Tw: 604 ± 38; AIN: Td: 349 ± 19; Tw: 373 ± 37. WB Zn/Hb:(µg/g): To: 32.6 ± 1.5; CG: Td: 51.1 ± 2.6; Tw: 45.1 ± 3.0; AIN: Td: 32.8 ± 1.1; Tw: 29.8 ± 2.2. RBC Zn/Hb:(µg/g): To: 29.5 ± 2.1; CG: Td: 48.1 ± 3.5; Tw: 41.9 ± 3.0; AIN: Td: 30.1 ± 1.2; Tw: 31.7 ± 2.5. In CG, WB Zn and WB Zn/Hb increased significantly at Td and Tw, regarding To. In AIN, WB Zn decreased but WB Zn/Hb and RBC Zn/Hb did not change. Taking into account the whole data, WB Zn/Hb correlated significantly with RBC Zn/Hb ($r = 0.98$; $p = 0.005$). The different patterns of Zn change in both groups indicate the need of a reformulation of the commercial diet.

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EFFECT OF ORGANIC SELENIUM ON T-2 TOXICOSIS IN CHICKENS

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It is believed that toxicity of mycotoxins is associated with their pro-oxidant properties and oxidative stress. The aim of this work was to assess effect of T-2 toxin on Se status and study effect of organic selenium in the form of Sel-Plex™ in combination with Mycosorb™ (a mycotoxin-binder) on T-2 toxicosis in chickens. Four groups of 5-day old chicks were formed with 20 birds per group. The control group was fed on a basal commercial diet. The experimental groups were fed on the basal diet with T-2 toxin added (8.1 mg/kg feed); T-2 toxin + Mycosorb™ , 1 g/kg feed; T-2 toxin + Mycosorb™ + Sel-Plex™ (0.3 ppm Se). After 3 weeks of feeding (26 days old) all birds were sacrificed and samples for biochemical analyses were collected. Inclusion of T-2 toxin in the chicken diet was associated with a significant ($P < 0.05$) decrease in Se concentrations in the liver (by 22.2%). Inclusion of Mycosorb into the chicken diet contaminated with T-2 toxin restored Se levels in the liver. A combination of Sel-Plex™ and Mycosorb™ further increased Se level in the liver. T-2 toxin compromised antioxidant defences in chickens, including decreased vitamin E, carotenoids and reduced glutathione concentrations in the liver. A combination of Sel-Plex™ and Mycosorb™ significantly inhibited antioxidant depletion by T-2 toxin.

COMMERCIAL EVALUATION OF PRODUCTION OF SE-ENRICHED CHICKEN

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While there is no simple solution for global Se deficiency, the poultry industry can make a valuable contribution through production of Se-enriched meat. The aim of the present work was to evaluate possibilities for enrichment of chicken meat with selenium and vitamin E under commercial conditions in the Ukraine at the RozDon company. The results indicated that dietary inclusion of organic Se in the form of Sel-Plex™ (Alltech Inc., USA) at 0.6-0.8 ppm Se from day old to slaughter significantly increased selenium level in the breast (from 85.2 to 284.3 ng/g) and leg (from 72.2 to 274.2 ng/g) muscle in comparison to the chickens fed a commercial diet supplemented with 0.1-0.2 mg Se/kg from selenite. Increased dietary vitamin E (250-500 mg/kg) during the last four weeks of growth also significantly increased vitamin E concentration in muscle tissue. A combination of dietary selenium and vitamin E was associated with a significant ($P < 0.01$) decrease of lipid peroxidation in the meat during storage at 4°C and at – 20°C. Our data indicate that consumption of approximately 100 g Se-enriched chicken meat, which can be produced commercially, could deliver about 50% of the RDA for Se and could help in solving problems of Se deficiency.

ORGANOSELENIUM (SEL-PLEX®) SUPPLEMENTATION TO MALE CHICKENS IMPROVES SPERMATOZOAL INTEGRITY

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Sodium selenite (SE) has been the traditional source of selenium in poultry diets, but selenium yeast (Sel-Plex® [SP]-organoselenium as selenomethionine in yeast protein) has become widely used in several countries signaling its importance as a substitute for selenite. SP is equivalent or even superior to selenite in terms of gut absorption, performance, induction of whole body feathering, and tissue retention. Therefore, it was of interest to extend our understanding of the influence of selenium on performance characteristics and investigate the influence of SE or SP in broiler breeders. Males were given diets that contained a grain-based background level

of 0.28 ppm of selenium (control) to which either SE or SP was supplemented at 0.2 ppm. Selenium supplemented roosters produced semen at 19 weeks of age while selenium-deficient roosters (control) did not produce semen until 26 weeks of age. Semen quality was best for SP-fed roosters with SE-fed roosters producing semen with quality intermediate between SP and selenium-deficient roosters. SP-fed males produced semen with the least number of abnormal sperm followed by SE and then no supplemental selenium. Organoselenium in yeast protein committed germ cells in the seminiferous tubules to produce sperm. Organoselenium in Sel-Plex® was the preferred form of selenium for the avian male.

Funding

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THE EFFECT OF PHYTASE ON THE BIOAVAILABILITY OF ZINC IN PIGLETS

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The effect of phytase from two sources on the bioavailability of Zn was investigated in 48 crossbred piglets, which were subdivided into 4 experimental groups. Animals in group 1 were fed a Zn deficient diet (29.7 ppm Zn/kg). In group 2 this basal diet was supplemented with 100 ppm Zn ($ZnSO_4$), in group 3 with 750 U/kg of phytase Ronozyme (CT) and in group 4 with 500 U/kg of phytase SP1002 (EX). In a first 22-day period, the animals of groups 2 to 4 were pair fed to the ad libitum fed group 1. In a following 21-day period, the animals of group 1 were switched to the diet of group 2 and feed was offered ad libitum to all animals. Pure zinc deficiency (group 1) significantly depressed apparent digestibility of dietary zinc, gain to feed ratio, plasma Zn content and plasma alkaline phosphatase activity. Those signs of zinc deficiency were significantly lessened when phytase of either source was included in the diet. However, especially when fed under ad libitum conditions, phytase supplementation did not totally compensate zinc deficiency when compared to animals of group 2.

Funding

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BIOPLEX MINERAL INCLUSION IN A 50% SKIM CALF MILK REPLACER ON CALVES PERFORMANCE

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The inclusion of protected minerals in the diet has a positive effect on the regeneration of ovarian and mammalian tissue. Forty Friesian male calves were used in an experiment to determine the effect of the inclusion of proteinated minerals in a 50% skim calf milk replacer (CMR) on the incidence of disease, feed intake and liveweight gain. The proteinated minerals consisted of Bioplex Cu (16 g/tonne), Bioplex Zu (37 g/tonne) and Bioplex Mn (25 g/tonne). Calves (mean initial weight 49 kg) were allocated at random to two isocaloric isonitrogenous CMR treatments as follows: standard - skim based CMR (C), standard fortified with proteinated minerals (B). Each calf received an allowance of 25 kg of milk reconstituted and offered warm during the 42-day experimental period. Calves were individually penned on straw and had ad libitum access to a 180 g/kg crude protein concentrate diet throughout. The data was subjected to one way analysis of variance. Incidence of respiratory disease was 30% and 35% for treatments C and B respectively. The corresponding values for liveweight gain (g/d) were 500 and 560 (sem 58) and for concentrate dry matter intake (kg) were 19.8 and 22.5. It is concluded that the inclusion of bioplex minerals to a skim based calf milk replacer increased concentrate dry matter intake by 2.7 kg and liveweight gain by 60 g/day.

BIOPLEX MINERAL INCLUSION IN A WHEY-BASED CALF MILK REPLACER ON CALF PERFORMANCE

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It has been reported that proteinated minerals had a positive effect on the regeneration of ovarian and mammalian tissue. Sixty-eight Friesian male and 12 Limousin x female calves were used in an experiment to determine the effect of the inclusion of proteinated minerals in a whey-based calf milk replacer (CMR) on the incidence of disease, feed intake and liveweight gain. The proteinated minerals consisted of Bioplex Cu (16 g/tonne), Bioplex Zu (37 g/tonne) and Bioplex Mn (25 g/tonne). Calves (mean initial weight 49 kg) were allocated at random to two isocaloric isonitrogenous CMR treatments: standard - whey-based CMR (C), standard fortified with proteinated minerals (B). Each calf received an allowance of 25 kg of milk reconstituted and offered warm during the 42-day experimental period. Calves were individually penned on straw and had ad libitum access to a 180 g/kg crude protein concentrate diet throughout. The data was subjected to one way analysis of variance. Incidence of respiratory disease was 50% and 25% for treatments C and B treatments respectively. The corresponding values for liveweight gain (g/d) were 640 and 650 (sem 54) and for concentrate dry matter intake (kg) were 20.4 and 21.6 (sem 0.22). It is concluded that the inclusion of bioplex minerals to a whey-based CMR significantly reduced the incidence of respiratory disease and did not affect liveweight gain or concentrate intake.

TRANSMIGRATION OF NEUTROPHILS AND DISTRIBUTION OF JUNCTION PROTEINS WERE AFFECTED BY ZINC DEFICIENCY IN CACO-2 CELLS

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Fundamental for the host defence is the capacity of neutrophils to migrate from blood vessel to inflammatory sites. During the migration across epithelial monolayer the cellular junctions are temporarily opened. Zinc deficiency can induce several alterations to immune response. In this study we have investigated whether zinc deficiency affected neutrophil transmigration and the cellular junction proteins. Caco-2 cells grown in complete medium (C cells), zinc depleted medium (Zn^- cells) or zinc repleted medium (ZnR cells) were used. The results indicate that the neuthophil migration across an inverted monolayer of cells, measured by myeloperoxidase activity, occurred earlier in Zn^- cells compared to C and ZnR cells, although the total amount of neutrophils transmigrated did not changed. The transepithelial electrical resistance showed a small decrease in Zn^- cells, but the paracellular mannitol passage was unchanged, indicating no damage in cellular junctions. The Zn^- cells appeared morphologically bigger and/or with irregular shape compared to C and ZnR cells. The distribution of Z0-1, occludin and catenin and the localization of E-cadherin were affected by zinc deficiency.

EFFECT OF ZINC SUPPLEMENTATION ON IMMUNE RESPONSE AND OXIDATIVE STRESS IN ITALIAN OLD POPULATION. THE ZENITH STUDY

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Zinc is fundamental for the activity of the immune system and for the antioxidant cell defense. Inadequate zinc intakes are often seen in the elderly population that may lead to a marginal zinc deficiency and consequently to an impairment of the immune response and inability to counteract oxidative stress. The aim of this study was

to investigate whether a supplementation of zinc could improve the immune response and antioxidant defense in a very old population. In this study 96 volunteers (> 70 years old), men and women, were divided in 3 groups receiving either 15, 30 mg Zn/day or placebo for 6 months. This was a 6-month placebo controlled double-blinded intervention study design. Zinc intake was evaluated by 4-day dietary record. Serum zinc level was evaluated before and after zinc supplementation. As markers of the immune response, the proliferative response of lymphocytes to a common polyclonal antigen and expression of cytokines of stimulated and non-stimulated lymphocytes were studied. As markers of the antioxidant defense, superoxide dismutase, glutathione peroxidase and catalase activities were analyzed. The results show a high variability on both the immune markers and antioxidant enzyme activities. Some markers of immune function apparently are differently influenced by zinc on male and female of older individuals. No relevant changes were observed after zinc supplementation in all the parameters studied.

MATERNAL IRON DEFICIENCY DURING PREGNANCY IN THE RAT INDUCES HIGH BLOOD PRESSURE AND OBESITY IN HER OFFSPRING

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Fe deficiency is a common and serious nutritional disorder, with well-known and serious sequelae. What is less well recognised, however, is that the consequences can extend well beyond the period of deficiency and, indeed if it occurs during pregnancy, into the adult offspring. We have developed a rat model to study this interaction. Female rats were fed either a control or Fe deficient diet (FD) prior to and throughout pregnancy. Litters were culled to 8 and pups cross-fostered to control fed mothers. All pups were weaned onto control diet. At birth, pups from FD mothers were smaller but caught up by 6 weeks. BP in the male offspring from FD dams was higher than controls. Blood volume correlated with BP, and there were significant changes in electrolyte balance, even at 16 weeks postnatally. By 16 weeks lean body mass was decreased and fat/lean ratio increased in offspring from FD dams. The increase was greater at 24 weeks post-birth. Currently the cause of the altered body composition in the offspring in unknown, however the data suggest that altered fluid retention causes a decreased haematocrit with a consequent increase in BP. These data are particularly important since they suggest that maternal anaemia may be a causal factor in the development of adult disease in the offspring.

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INDUCTION OF THIOREDOXIN REDUCTASE IN BROILER CHICKENS FED ORGANOSELENIUM (SEL-PLEX®)

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Thioredoxin reductase (TrxR), a selenium-dependent enzyme, which belongs to a superfamily of flavoenzyme disulfide oxidoreductases, has been reported to increase its activity after selenium supplementation to animals as compared with enzyme activity in selenium deficient animals. TrxR activity (nmol NADPH/min/mg total protein) was determined in tissues from 3-week-old male broiler chickens fed supplemental levels of selenite (SE, 0.3 ppm), Sel-Plex® (SP, 0.3 ppm; a source of organoselenium in yeast protein), a combination of 0.15 ppm selenite + 0.15 ppm Sel-Plex® (SS), or no supplemental selenium (negative control, 0 Se). The subcellular distribution of TrxR in chicken hepatic cells showed the highest activities in decreasing order in the nuclear pellet, the mitochondrial lysate, post-mitochondrial supernatant, mitochondrial membranes, post-nuclear supernatant, and the mitochondrial pellet. In heart, brain, breast muscle, bursa of Fabricius, thymus, and spleen, the feeding of SP elevated TrxR activity, but in red blood cells, liver, lungs and kidney, selenium sources did not alter TrxR activity. Feeding SE at 5, 10, or 15 ppm caused a plateau in TrxR activity at 10 ppm, but even at 15 ppm TrxR activity in SP-fed broilers was still increasing. The results show that chicken TrxR is induced by selenium and with Sel-Plex® the induction continues through very high levels of supplementation.

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SELENIUM PROTECT AGAINST CADMIUM INDUCED DNA DAMAGE – IN VITRO STUDY

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Toxic effects of heavy metals on cells involve among others generation of reactive oxygen species, disturbance of antioxidative processes and damage of lipids, proteins and DNA. Cadmium shows affinity to thiol groups, and the decreased concentration of intracellular glutathione is most likely the underlying cause of oxidative stress. Selenium is a trace element involved in antioxidative processes and heavy metal detoxication, through activity of glutathione peroxidases, tioredoxin reductases, selenoprotein P etc. The aim of this study was to investigate the protective effect of selenium on cadmium induced DNA damage. The mice fibroblasts (WEHI 164) were cultured in medium with low (0. 15 ng/ml), standard (1.5 ng/ml) and high selenium (50 ng/ml) concentration and stressed with 1,5 μg $CdCl_2$/ml medium for 24 h. Oxidative DNA damage measured by the percent of DNA in comet tail and by fpg sites, was lower in cells with standard and high selenium medium as compared with cells growing in low selenium medium ($p < 0.05$). Incubation cells with cadmium alone or Cd in the presence of H_2O_2 induced statistically significant oxidative DNA damage ($p < 0.05$). Percent DNA in comet tail in cadmium treated cells was inversely correlated with selenium concentration in medium. Differences were not found in DNA damage in high Se cells treated with Cd and controls (without Cd) – Se supplementation protected against cadmium-induced DNA damage.

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CALCILIFE PROVIDES ENHANCED NUTRITION AND INGREDIENT FUNCTIONALITY

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Bone mineralization in growing rats requires an adequate supply of minerals in the diet. Mineral absorption may be affected by the dietary mineral source and solubility. Calcilife is a new ingredient combining a natural, sea plant derived mineral source high in calcium, magnesium and other bone essential trace minerals with the calcium-absorbing powder of short chain fructooligosaccharides (scFOS). Thus the addition of scFOS to Aquamin (Calcilife) may increase the amount of mineral incorporated into bones of growing rats. Thirty-two 4-week old male Sprague-Dawley rats were divided into four treatment groups of control (calcium carbonate), Aquamin, $CaCO_3$ + scFOS and Calcilife diets. At week 12, rats were sacrificed and bones were removed for analyses. Total bone mineral concentration was not affected by diets for any bone. However, bone mineral content confirms the beneficial effect of Calcilife. Rats fed Aquamin diets had higher magnesium concentration and total magnesium per bone in the femur, tibia and humerus. Aquamin containing diets resulted in higher tibia dry weights and higher total calcium, iron, zinc and phosphorous per bone. Calcilife increased femur zinc/bone (10%) and magnesium/bone (21%) when compared to controls. Rats fed Calcilife had higher vertebral BMD than rats consuming other diets. The synergistic effects of Calcilife in diets increased bone mineralization and density and would be beneficial as a nutraceutical supplement.

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INFLUENCE OF AGE ON ANTIOXIDANT ENZYME ACTIVITY IN THE PIG

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Humans and livestock manage potential cell damage by cellular pro-oxidants by utilizing antioxidant enzymes and molecules. While aging has been associated with irreversible oxidative damage to cells, little is known

about the influence of age in the growing animal on glutathione peroxidase (GPx-1), Cu/Zn superoxide dismutase (Cu/Zn SOD) and catalase (CAT) activity. Our objective was to evaluate these enzymes at four periods in the pig's life: at birth (0 d - 14 ± 1 h), the suckling period (13 d of age), the growth period (54 ± 3 d), and around puberty (approximately 180 d). In addition, hepatic mineral concentrations were determined. Hepatic Cu concentrations increased from birth to 13 d of age, but decreased from 13 to 180 d (582.63 and 820.80, vs. 184.48 and 104.56 μmol/kg, respectively; $p = 0.0001$). Liver Zn, Mg, Mn, Ca and P concentrations increased ($P < 0.001$) with age. Newborn pigs had less GPx-1 activity than older pigs (0.49 vs. 0.64, 0.71, 0.79 U/mg protein; $p = 0.01$) and 13 d old pigs had less activity than 180 d old pigs. Hepatic cytosolic Cu/ZnSOD activity was lower in newborn than in older pigs (24 vs. 40, 43, 61 U/mg protein; $p = 0.0001$) and 180 d old pigs had greater activity than 13 and 54 d old pigs. Hepatic CAT activity increased as age increased (0.003, 0.020, 0.044, 0.061 k/mg protein; $p = 0.0001$). Our results indicate that as pigs age, they are able to increase antioxidant activity with adequate nutrition.

MICRONUTRIENT DEPRIVATION INCREASES AMINO ACID TRANSPORT SYSTEM A ACTIVITY IN THE BEWO CHORIOCARCINOMA CELL LINE

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Amino acid transport system A is regulated by many factors and increased activity may represent a generalised cell stress response. Iron deficiency induces a response similar to that of hypoxia and copper deficiency reduces the cells capacity to manage free radicals, inducing an oxidative stress response. If increased system A activity is a generalised stress response, then you would expect micronutrient deficiency to lead to increased system A activity. We studied the response of system A to iron and copper deficiency in BeWo cells. BeWo cells were obtained from Dr A L Schwartz (St. Louis, Missouri, USA) and routinely maintained in Dulbecco's Modified Eagle Medium + Glutamax 1 + 10% fetal calf serum and 2% penicillin/streptomycin. Iron deficiency was induced by incubation with 20 μM Desferrioxamine for 40 h and copper deficiency by incubation with 20 μM Diamsar for 18 h. System A activity was investigated using transcellular transport studies with 14C-MeAIB and polarised cells on bicameral filters. Incubation of cells in iron or copper deficient media significantly ($P < 0.05$) increased the transcellular transfer rate of 14C-MeAIB compared to time matched controls. The increases in transfer rate were reversible when micronutrients were replenished. This is the first study to show that altered micronutrient status regulates amino acid transport system A and supports the hypothesis that increased system A activity is a general stress response.

Funding

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THE EFFECT OF AN INTRA-VENOUS INFUSION OF THIOMOLYBDATE ON COPPER STATUS AND OVARIAN UNCTION OF SHEEP

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The contentious issue of whether thiomolybdates are taken up into the blood of ruminants was bypassed by infusing tetrathiomolybdate intravenously into ewes with ovarian autotransplants, a technique that allows repeated collection of ovarian venous blood in conscious animals. Oestrous cycles were synchronised and animals infused with either tetrathiomolybdate (1 mg/h, $n = 6$) or saline ($n = 6$) for 5 days, from 2 days prior to the induction of luteal regression and for 3 days over the subsequent follicular phase. Blood samples were collected for copper analysis (plasma copper-PlCu, serum caeruloplasmin-CP, CP:PlCu ratio-ratio, TCA insoluble copper-TCA) and reproductive (luteinising hormone, follicle-stimulating hormone, oestradiol progesterone) status. The thiomolybdate infusion did not significantly alter the overall PlCu or CP but PlCu

increased in treated ewes especially towards the end of the infusion and CP decreased, resulting in a significant treatment-time interaction ($P < 0.05$). Similarly ratio was reduced ($P < 0.05$) and TCA increased ($P < 0.05$) over the infusion period. Two of the 6 treated ewes failed to ovulate and pre-ovulatory oestradiol, LH and FSH and post-ovulatory progesterone concentrations were reduced. In conclusion, infusion of a physiological dose of thiomolybdate markedly altered copper status and led to a depression in ovarian function. Further work is needed to confirm effects of treatment on ovarian hormone levels.

CONNECTION OF HAIR ELEMENTAL CONTENT WITH SOME ANTHROPOMETRIC PARAMETERS IN CHILDREN OF THE FIRST YEAR OF LIFE

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In this study data on chemical elements content of children's hair (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, P, Pb, Se, Si, Sn, V, Zn) was compared with some body constitutional parameters widely used in medical and hygienical practice. The results revealed the existence of significant correlations between content of some elements in hair of children under one year and their body height and weight. The closest connections with anthropometric parameters were characteristic of Ca, Mg, Fe and Co hair content. Significant positive correlations were found for these elements with body weight ($r = +0.75$, $+0.55$, $+0.67$, $+0.62$, accordingly) ($p < 0.01$), and with body height ($r = +0.57, +0.45, +0.77, +0.62$, accordingly) ($p < 0.01$). It apparently reflects direct participation of these chemical elements in the processes connected to growth of the organism such as protein synthesis, development of bone and cartilaginous tissue, formation of immune system, CNS, locomotorium, skin and mucosae. Thus, optimal supply of these elements conduces to proper growth and development of children. It was found that girls were featured by more distinct correlations of anthropometric parameters with the content of essential elements as compared to boys. Boys were featured by stronger dependence of growth processes on content of toxic and conditionally toxic elements in the organism, which was reflected in higher correlation of body height with As ($r = +0.50$), Hg ($r = +0.55$) and V ($r = +0.33$) ($p < 0.01$). Perhaps it is connected with increase of these elements content in hair due to their elimination from the organism. As for the other trace elements, despite the high statistical significance the correlation coefficients were found to be very low not allowing us to say about existence of any dependencies.

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SELENIUM SUPPLEMENTATION AND OUTCOME IN SEPTIC ICU

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As a result of recent evidence (Angstwurm et al. 1999) documenting the improved clinical outcome with selenium (Se) replacement in septic patients, 40 ICU patients with an APACHE II (Acute physiological and chronic health evaluation score is used to assess the severity of illness in critically ill patients) score >15 were randomly stratified to receive Se intravenously either a high dose [6, 4 & 2 μmol/day, each for 3 consecutive days, group Se +, n = 18], or standard dose [0.4 μmol of Se group Se-, n = 22]. Plasma Se, glutathione peroxidase (GPx), F2 isoprostanes and RBC GPx were estimated on day 0, 3, 7, 14. Clinical outcome markers included SOFA score, 28-day mortality, duration of ICU stay, infection rate and renal replacement therapy. Results showed a significant increase in plasma Se and GPx levels in Se+ as compared to Se- group. However there was no significant difference in mortality rate, renal replacement therapy, infection rate or duration of stay in ICU between two groups. Our study was unable to confirm the previous suggestion that an increased dose of Se improves outcome in critically ill patients.

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COMPARISON OF SERUM SELENIUM CONCENTRATIONS OF GESTATIONAL DIABETIC WOMEN, NON-DIABETIC PREGNANT WOMEN AND HEALTHY CONTROLS IN HUNGARY

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In recent years a decrease in plasma selenium during pregnancy has been associated with glucose intolerance. Low selenium status has also been indicated to be a risk factor for pre-eclampsia (Rayman et al. 2003). The Hungarian population has been shown to have relatively low serum selenium levels but the selenium status of Hungarian pregnant women has not yet been investigated. The serum selenium levels of 15 gestational diabetic pregnant women, 15 non-diabetic pregnant women and 15 healthy controls were compared, as well as their hsCRP values and lipid parameters. The blood was taken between the 24th and the 28th week of pregnancy when the glucose load was performed. Selenium was determined via atomic absorption spectrometry following hydride generation. HsCRP was measured by immunturbidimetry. The serum selenium concentrations of the gestational diabetics and non-diabetic pregnant women were 45.9 ± 12.1 μg/l and 40.5 ± 8.0 μg/l, respectively. Healthy controls had significantly higher serum selenium concentrations of 81.9 ± 14.2 μg/l ($p < 0.01$ compared to gestational diabetics and $p < 0.001$ compared to non-diabetic pregnant women). Cholesterol, HDL, LDL and triglyceride concentrations were higher in pregnant women compared to controls with no difference between the two pregnant subgroups. HsCRP values of both gestational diabetic and non-diabetic pregnant women were higher than in controls ($p < 0.01$). Conclusions: Both gestational diabetic and non-diabetic pregnant women had significantly lower serum selenium concentrations than the healthy non-pregnant controls. The higher hsCRP values in pregnant women may be explained by increased lipid peroxidation during pregnancy. Pregnant women in Hungary have low serum selenium concentrations and selenium supplementation should definitely be recommended for them.

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COMPARISON OF SERUM SELENIUM CONCENTRATIONS OF GESTATIONAL DIABETIC WOMEN, NON-DIABETIC PREGNANT WOMEN AND HEALTHY CONTROLS IN HUNGARY

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In recent years a decrease in plasma selenium during pregnancy has been associated with glucose intolerance. Low selenium status has also been indicated to be a risk factor for pre-eclampsia (Rayman et al. 2003). The Hungarian population has been shown to have relatively low serum selenium levels but the selenium status of Hungarian pregnant women has not yet been investigated. The serum selenium levels of 15 gestational diabetic pregnant women, 15 non-diabetic pregnant women and 15 healthy controls were compared, as well as their hsCRP values and lipid parameters. The blood was taken between the 24th and the 28th week of pregnancy when the glucose load was performed. Selenium was determined via atomic absorption spectrometry following hydride generation. HsCRP was measured by immunturbidimetry. The serum selenium concentrations of the gestational diabetics and non-diabetic pregnant women were 45.9 ± 12.1 μg/l and 40.5 ± 8.0 μg/l, respectively. Healthy controls had significantly higher serum selenium concentrations of 81.9 ± 14.2 μg/l ($p < 0.01$ compared to gestational diabetics and $p < 0.001$ compared to non-diabetic pregnant women). Cholesterol, HDL, LDL and triglyceride concentrations were higher in pregnant women compared to controls with no difference between the two pregnant subgroups. HsCRP values of both gestational diabetic and non-diabetic pregnant women

were higher than in controls ($p < 0.01$). Conclusions: Both gestational diabetic and non-diabetic pregnant women had significantly lower serum selenium concentrations than the healthy non-pregnant controls. The higher hsCRP values in pregnant women may be explained by increased lipid peroxidation during pregnancy. Pregnant women in Hungary have low serum selenium concentrations and selenium supplementation should definitely be recommended for them.

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MULTIELEMENT HAIR PROFILE (MHP) FOR EARLY DETECTION OF BONE OSTEOPOROSIS IN MEN AND WOMEN

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The aim of this study was to show how the MHP "chemical portrait" may help in early diagnose of osteoporosis. Case #1. The MHP of a men 65 years old showed a mild increase in Ca (1.6^0), Mg (1.6^0), and Si (1.2^0) content according to the CBM scale. Normal values are ± (≤1.00). However, his DEXA bone mineral density T scores of lumbals spine and at the neck of the left femur were -0.5 and -0.3, respectively. The DEXA values are considered to be normal in up to -1.0. Male osteoporosis. Case #2. The MHP of a women 47 years old showed an appreciable increase in Ca (2.0^0) and Mg (2.0^0). Her Ultrasound Bone Densitometer (UBD) findings were normal, whereas her overall DEXA lumbal Tscore was decreased (-1.23); L1 and L2 were within the normal range, whereas L3 and L4 were low. Female osteoporosis. In Case #1, MHP of an old adult man with no clinical bone problems showed an increased loss of principal bone minerals (Ca, Mg, and Si), whereas the DEXA findings were still considered normal. In Case #2 the UBD was normal whereas DEXA showed signs of osteoporosis. This finding indicates that detection of increased Ca and Mg (and perhaps Si and Sr) concentrations in the hair is an early warning sign of osteoporosis in men and women. This indicates that in men, osteoporosis can be detected by MHP even before it it detected by DEXA diagnostic criteric. In contrast to the metabolically active "equilibrating" of the bone, hair is the "memory tissue", a longitudinal integral of metabolic activity over the time. The results support the view that osteoporosis is an endogenous, irreversible demineralization process with Ca loss as the most characteristic feature.

MULTIELEMENT HAIR PROFILE (MHP) IN A MAJOR CLINICAL DEPRESSION (MCD) INDICATES A STRONG ETIOLOGICAL LINK TO THE EUTHYROID IODINE DEFICIENCY (ID)

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We studied the MP of 41 element in the human scalp hair of 29 subjects with MCD (16 women, 13 men) and 20 healthy controls (C) (15 W, 5 M), 23 to 64 years old (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Ge, Hg, I, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, Rb, Sb, Se, Si, Sn, Sr, Ti, Tl, V, W, Zn, Zr). The MCD was verified with Beck Inventory Depression Scale; the Declaration of Helsinki was strictly followed. Individual hair samples were cut, stirred 10 min in anethyl ether/acetone (3:1 ww), dried at 85ºC/1 h, immersed 1 h in 5% EDTA, rinsed in re-distilled H_20, dried at 85ºC/12 h, wet digested in HNO_3/H_2O_2, and analyzed for MP with ICP-MS (Elan 2000, Perkin-Elmer, Uberlingen, Germany). The chemicals were pro analyis grade (Khimmed Sintez, Moscow, Russia). We used Seronorm Trace Elements lyopilized reference material (SERO AS, Bilingstadt, Norway). Overt trace element deficiency and/or excess (D/E) was observed for 13 elements (underlined elements), sporadic D/E was observed for 13 elements (dotted), and the rest of the elements show no D/E. Our principal finding was the high incidence of ID in MCD ($p<0.05$ vs C); frequently associated with Se and Cu deficiency (normal hair I = 0.5 - 2.8, Se = 0.8 - 3.0, and Cu = 0.8 - 1.3 mg kg1) . Intriguing MP patterns were

observed for the K/Na and Co/Mn elemental pairs, and Fe/Cr/Zn, and Ca/Mg/Si triplets. The results strongly suggest an associative/causative link between the ID and/or iodine related euthyroid metabolic disorder in the etiology of MCD.

HUMAN BLOOD MOLYBDENUM; COMPARISON OF DIFFERENTIAL PULSED ANODIC STRIPPING VOLTAMMETRY (DPASV), THERMAL EMISSION ATOMIC ABSORPTION SPECTROMETRY (ET-AAS), AND INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

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We compared the results of our recently developed DPASV method for the analysis of the human whole blood molybdenum (HWB Mo) with ET-AAS and ICPMS, in a double blind multicentric quality control study. Ten registered nurses donated blood samples; the recommendations of the Helsinki Declaration were strictly followed. The coded blood vials (BD Vacutainer®, Beckton Dickinson, Plymouth, UK), were randomly allocated for the analysis: (1) DPASV (Central Unit μAutolab type II, Eco Chemie, Utrecht, The Netherlands; Electrode Unit 663 VA Stand Metrohm, Harisau, Switzerland), (2) ETAAS (AAnalyst 600, Perkin Elmer Instruments, Shelton, CT, USA), and (3) ICPMS (Elan 2000, Perkin-Elmer, Uberlingen, Deutchland). Blood for DPASV and ETAAS was dried at 105ºC, ashed at 450ºC/24 h, dissolved in 0.25 ml conc HNO_3 and made up to 5.0 ml with re H_2O. The chemicals were pro analysi (Suprapur grade, Merck, Darmstadt, Germany). Blood for the ICP MS (0.5 ml) was digested in a microwave oven with 0.1 g of HNO_3 (Khimmed Sintez, Moscow, Russia) at 175ºC/20 min. We used Seronorm Trace Elements lyopilized HWB reference material (SERO AS, Bilingstadt, Norway). There was no difference in the HWB Mo results between the DPASV, ETAAS and ICPMS, or in-between any two of them (ANOVA, $P > 0.05$). The all results data distribution ($n = 30$) was highly skewed to the right (mean of 2.55 μg Mo L-1); the median of 1.76 μg Mo L-1 reflected better the data clustering.

EFFECT OF ORGANIC SELENIUM (SEL-PLEX) IN COMBINATION WITH A-TOCOPHEROL ON MEAT STABILITY OF FRESH AND FROZEN POULTRY MEAT

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The objective of this research was to determine the antioxidative effect of Se dosed as dietary organic (Sel-Plex®) in combination with α-tocopherol (100 IU/kg feed) on poultry breast and thigh. Birds were supplemented with 0, 0.05, 0.10 and 0.30 ppm Se from Sel-Plex® with or without 100 IU α-tocopherol per kg of feed for 42 days (6 birds/treatment). At 42 days, broiler breast and thigh muscle were homogenized, partly used for the immediate determination of TBARS (Thiobarbituric acid value) (expressed as mg Malonaldehyde (MDA)) (kg meat), while the remainder was packed in closed plastic containers and kept at –20°C for 16 weeks after which time TBARS were determined. The results on fresh meat samples indicated that by supplementation of 0.05 ppm Se the MDA value was reduced significantly ($P < 0.01$) in the breast and tight muscle to values which were very similar to the treatment with 100 IU α-tocopherol and no added Se. Supplementation of α-tocopherol in combination with 0.05 ppm of Se reduced further MDA levels in breast (not significantly) and thigh (significantly, $P < 0.05$). Increasing levels of Se up to 0.3 ppm reduced TBARS in breast and thigh muscle further while at these levels the supplemental α-tocopherol did not have any surplus effect. The results of the TBARS on breast and thight muscle after storage under frozen conditions demonstrate that 0.1 ppm Se was required to obtain a similar or better antioxidative effect as 100 IU/kg α-tocopherol. Also, increasing the Se concentration up to 0.3 ppm reduced TBARS significantly ($P < 0.05$) compared to the 0.1 ppm Se level, and adding α-tocopherol on top of the Se supplementation had in breast muscle a non-significant ($P > 0.05$), yet in thigh muscle a significant ($P < 0.05$) effect on MDA levels.

REPLACEMENT OF INORGANIC CU, MN, FE AND ZN WITH BIOPLEX® ON GROWTH PERFORMANCE AND FAECAL MINERAL EXCRETION IN BROILERS

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In a broiler study, a total of 21 floor pens each with 40 birds per pen were used, 7 replicates per treatment. Three treatments were imposed: (A) control feed without added minerals, (B) control feed with added Cu to equal an addition of 12.5 ppm in the feed and added Mn, Zn and Fe to equal 40 ppm in the feed; all inorganic sources and (C) equal to treatment B but using Bioplex® Cu, Fe, Mn and Zn; organic source. Measurements of body weight gain, feed conversion ratio and hock score were taken at 49 d of age, faecal samples were collected at day 35 (10 per treatment) for analysis of minerals. Litter was scored at 21 and 42 days. The results indicate that body weight and feed conversion at 49 were not significantly different between treatments. Assuming that the mineral excretion in treatment B and C of the minerals already present in the feed (17.4, 74.2, 67.9 and 169.3 mg/kg of Cu, Mn, Zn and Fe respectively) was equal to treatment A, it could be calculated that the reduction in output of these minerals by either supplying minerals in the organic (Bioplex®) or the inorganic from was 17.0, 71.4, 48.8 and 105.2 mg/kg DM for Cu, Mn, Zn and Fe respectively, equating to a reduction in excretion of 41.3, 49.8, 36.5 and 50.3% of added minerals. Litter score and Hock were not statistically different between treatments, however the Hock score was lower for the broilers fed on Bioplex® compared to the control and the broilers fed added inorganic minerals (75.0 versus 77.9 and 70.7 for treatment A, B and C respectively).

INHIBITION OF F9 CELL PROLIFERATION BY DESFERRIOXAMINE IS REVERSIBLE AND IS INDEPENDENT OF IRON REGULATION.

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The iron-chelator desferrioxamine (DFO) inhibits cell proliferation and induces cell differentiation in the F9 (mouse teratocarcinoma) stem cell model (Tanaka et al. 1999). However, whether it works through iron deprivation or some other mechanism is not known. If the former, it would be expected that Fe-dependent gene expression would increase. Cell proliferation was determined using Almar Blue conversion by F9 cells cultured under standard conditions in gelatine-coated 96-well plates. For gene-expression studies, F9 cells were cultured in uncoated Petri dishes and RNA isolated using the TriReagent™ protocol prior to Northern blot analyses. All experiments were performed in triplicate. Results: DFO inhibits cell proliferation in a dose-dependent manner. The inhibitory effect is reversible within 48 h for low, but not high, levels of DFO exposure. In control cells, gene expression of iron transporters (e.g. transferrin receptor, TfR) increased steadily over time, but there was no relative augmentation of TfR mRNA in cells exposed to increasing [DFO]. Furthermore, there was no difference in the TfR gene expression profiles following reversal of DFO exposure. Thus, we can conclude that DFO inhibition of F9 cell proliferation is reversible and, importantly, seems to be independent of iron regulation.

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SUPPLEMENTATION OF BROILER BREEDER DIET WITH ORGANO-SELENIUM COMPOUNDS INCREASES SELENIUM CONCENTRATION IN THE EGG MEMBRANES AND SHELL

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Glutathione peroxidase (GSH-Px) is considered to play an important role in the antioxidant protection of egg yolk and albumen (Gaal et al. 1995). Consequently manipulation of dietary selenium can affect the concentration of selenium in the eggs components, providing protection against the damaging effects of free radicals. Broiler breeders (N = 160) were fed either a diet low in selenium (0.1 mg/kg) or the same diet with selenium added as Sel-Plex® (0.4 mg/kg). Eggs were collected and analysed for selenium. Selenium concentration in the shell, albumen, perivitelline membrane, yolk and shell membrane increased from 13, 30, 66, 85 and 131 ng/g for the non supplememted treatments to 77, 249, 262, 516 and 854 ng/g for the supplemented treatments respectively. The results indicate that by using dietary organo-selenium compounds it is possible to increase its concentration in various egg components and to potentially provide additional protection to the hatching egg and consequently the developing embryo.

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INVESTIGATING THE INHIBITORY EFFECT OF CALCIUM ON IRON UPTAKE AND TRANSPORT USING A CACO-2 TRANSWELL SYSTEM

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Single meal absorption studies demonstrate an inhibitory effect of iron on calcium absorption, but the mechanism is unclear. The aim of this study was to identify the site of the effect and its molecular control using the Caco-2 transwell model. The Caco-2 colorectal carcinoma cell line has physical characteristics that closely resemble intestinal epithelial cells when grown to confluence and, when cultured on a permeable membrane, undergo morphological and biochemical changes to form a polarized monolayer with a brush border on the apical surface and intercellular tight junctions. The transwell system was optimised using apo-transferrin to mimic the physiological conditions of cells in vivo and used to measure cellular uptake and iron transport. In the presence of calcium at levels equating to those found in the diet, we observed an inhibition of both iron uptake into the cell ($p < 0.01$) and transfer across the basolateral membrane ($p < 0.01$). The expression of key genes involved in iron transport is currently being measured using real time RT-PCR (Taqman) and results will be presented that provide further insight into the mechanism of inhibition of calcium.

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AN ORGANIC SELENIUM ADDITIVE (SEL-PLEX®) IN DAIRY COWS DIETS

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Twenty multi-parous cows were used to compare effects of an organic selenium (Se) additive (Sel-Plex®) with inorganic Se (Na selenite) on performance, and health in dairy cows. All cows received a total mixed ration (TMR) containing maize and grass silage, cracked wheat, soybean meal, rapeseed meal, and minerals, which provided dietary Se (mg/kg DM) values of 0.15 (T1 control no added Se), 0.27, 0.33, 0.41 (T2 to T4 Sel-Plex®) and 0.25 (T5 Na selenite). There were no significant treatment effects on DM intake, milk yield, milk composition, milk urea and somatic cell count (SCC) Blood chemistry and haematology showed few significant treatment effects. Milk and blood Se values (µg/l) for T1 to T5 were 19, 28, 41, 54 and 21; and 211, 214, 235, 251 and 208. Regression analysis of T1 to T4 noted significant ($P < 0.001$) linear effects of increasing dietary Se from Sel-Plex® for milk, blood, urine and faeces. Data analysis showed Sel-Plex® (T2) produced a significantly higher ($P < 0.05$) milk Se content compared with Na selenite (T5) (27.8 vs.20.8 mg Se/l), but not with blood, urine and faecal Se values. When compared with Na selenite, Sel-Plex® increased the concentration of selenomethionine in milk and blood. Cow health was unaffected by treatment and there was no indication of adverse effects associated with the use of Sel-Plex®.

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EFFECTS OF SOURCE AND LEVEL OF DIETARY COPPER ON COPPER AND ZINC METABOLISM

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Effects of dietary copper (Cu) source and level were evaluated using seven dietary treatments. Six birds were allocated to each of nine replicates per treatment based on weight at 7 days of age. The birds were housed in cages in an environmentally controlled building. Diets were fed from 7 to 21 days. Sources of Cu were Cu sulfate and chelated Cu (Bioplex™, Alltech, Nicholasville, KY). All diets contained 75 ppm added Zn from Zn sulfate. Cu sources were added to a basal diet (Trt 1): Cu sulfate at the levels of 62.5 ppm (Trt 2), 125 ppm (Trt 3), 250 ppm (Trt 4); Bioplex Cu at 25 ppm (Trt 5), 50 ppm (Trt 6), 100 ppm (Trt 7) dietary Cu. Cu content of the basal diet was 20 ppm. Feed consumption was depressed ($P < 0.05$) by the high level of Cu sulfate (Trt 4), resulting in an 18 g reduction in body weight. Body weight and feed consumption were not significantly different for the other treatments. Source or level of Cu did not affect feed conversion. Liver Cu levels were elevated with the high level of Cu sulfate. Excreta were collected for 14 days from two replicates per treatment. There was a linear increase in excreta Cu content as dietary Cu level increased. Cu retention decreased as the levels of Cu sulfate increased; conversely, retention of Cu from Bioplex Cu increased linearly as dietary levels increased and were on average 35% higher in the birds fed Bioplex Cu ($P < 0.01$). Zinc (Zn) retention was reduced as Cu sulfate levels increased and Zn retention was higher ($P < 0.03$) for birds fed Bioplex Cu. Liver Zn content was depressed with the highest level of Cu from Cu sulfate, however liver Zn was maintained with all levels of Bioplex Cu. These data suggest that using Bioplex Cu may reduce Cu-Zn antagonisms and increase the retention of both minerals.

NUTRITIONAL MEANS TO LOWER TRACE MINERAL EXCRETION FROM PIGS WITHOUT COMPROMISING PERFORMANCE

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Two replications of 264 pigs were used in a completely randomized design with four dietary treatments throughout the grow-finish period. Pigs were housed 10-12/pen (n = 24) and allotted by sex and randomly assigned to treatments at ~27 kg body weight (BW). Trt 1 (control) contained a commercial trace mineral supplement, which met or exceeded NRC nutrient requirements for swine (1998). Trts 2, 3, and 4 contained Bioplex™ (Alltech, Inc.) trace minerals at 100%, 75%, or 50% of the commercial supplement levels. Individual 10th rib backfat (BF) and loin muscle area (LMA) were measured ultrasonically prior to marketing (118 kg). Average daily gain and lean growth on test (LGOT) were calculated individually. Fecal samples were taken at each of the four diet phase changes. Pen was the experimental unit; and data were analyzed using a mixed linear model. Control pigs consumed less ($P < 0.05$) feed/day than pigs fed the test diets. There were no differences ($P > 0.05$) among treatments for BF, LMA, ADG, or LGOT. Control pigs excreted greater ($P < 0.05$) Cu concentrations across the four collection periods. During phase 3, pigs fed Bioplexes excreted less ($P < 0.05$) Cu than controls. Control pigs during phases 1, 2, and 3 excreted greater ($P < 0.05$) Fe concentrations than pigs fed test diets during collections 2, 3, and 4. Less Zn ($P < 0.05$) was excreted by pigs fed Trt 4. However, pigs fed Trt 1 had higher ($P < 0.05$) fecal Zn than pigs fed test diets over the four collection phases. Trt 4 (50% Bioplex™) most effectively lowered trace mineral excretion. These data show that feeding Bioplex™ organic minerals reduced Cu excretion of by 75%, Zn by 50%, and Fe by 14% without significantly compromising growth or carcass characteristics

NUTRITIONAL MEANS TO LOWER TRACE MINERAL EXCRETION FROM POULTRY WITHOUT COMPROMISING PERFORMANCE

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To evaluate the effects of reduced dietary trace mineral levels on growth and mineral excretion of broiler chickens, 246 Cobb X Cobb males were used in a randomized completed block design. Seven replicates of birds were blocked by room position and exposed to continuous lighting. There were seven dietary treatments. Treatments 1-3 contained a premix that provided 100, 50, and 25%, respectively of NRC (1994) requirements for Cu, Zn, and Fe supplied by sulfates. The NRC (1994) states the requirements for broilers from 1-3 weeks of age are 5 ppm Cu, 100 ppm Zn, and 30 ppm Fe. Treatments 4-6 contained a premix that contained 100, 50, and 25%, respectively, of NRC (1994) requirements for Cu, Zn, and Fe supplied by Bioplex™ organic minerals. Treatment 7 served as a negative control with no added trace mineral premix. Birds were housed in 35 × 54 cm cages with ad libitum access to feed and water. The experimental duration was 14 d. Birds and feed were weighed at the beginning and end of the experiment to determine gain, feed intake, and feed efficiency. On d 9-10 total excreta was collected to determine mineral content and retention. Data were analyzed as a randomized complete block design with pen serving as the experimental unit. There was a linear ($P < 0.01$) decrease in gain and feed intake for birds fed decreasing levels of inorganic minerals. In contrast, growth performance and feed intake were maintained as mineral levels were decreased in the Bioplex™ treatments. Excreta Cu and Zn concentrations decreased linearly ($P < .01$) as the dietary levels decreased regardless of source. These data suggest that only 25% of current NRC (1994) requirements for poultry may be needed for normal performance when fed as Bioplex™ minerals.

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IRON INTAKE AND IRON STATUS IN ADULT BELGIAN WOMEN

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Adult women (18-39 years) from the region of Ghent were examined in 2002 to describe and analyse their iron intake and iron status. Iron intake was determined by a computerised iron intake assessment tool. To determine the iron status different biomarkers were measured. Pearson correlation was used to evaluate the association between intake and status. The mean (± S.D.) iron intake was 10.4 (± 3.33) mg/day (n = 796). Haem and non-haem iron intake were respectively 0.7 (± 0.50) and 9.6 (± 3.22) mg/day. 99% had an iron intake below the Belgian RDA (20 mg/day). The foods with the highest mean proportional contribution to total iron intake were: bread (19.3%), meat (12.3%), cereals (11.7%) and vegetables (10.8%). The means (± S.D.) for the iron status biomarkers were: 13.5 (± 1.00) g/dl haemoglobin, 40.9 (± 3.62)% haematocrit, 4.5 (± 0.49) x 106/µl RBC, 99.0 (± 41.62) µg/dl serum iron, 2.8 (± 0.50) g/l transferrin, 37.9 (± 35.11) ng/ml ferritin and 1.2 (± 0.39) mg/l sTfR (N = 778). Iron deficiency (ferritin < 12 ng/ml and haemoglobin > 12 g/dl) was seen in 13.5% of the women and iron deficiency anaemia (ferritin < 12 ng/ml and haemoglobin < 12 g/dl) in 2.1%. Pearson correlation coefficient for iron intake and ferritin was 0.031 and for iron intake and sTfR -0.019 (n = 736). In conclusion: the iron intake in adult Belgian women is considerably lower than the current recommendations. However, this does not reflect in the iron status, for which better results are seen. Moreover, no correlation was found between iron intake and iron status.

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NOVEL THIOARSENIC METABOLITES IN HUMAN URINE AFTER INGESTION OF AN ARSENOSUGAR

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Arsenic compounds are common constituents of seafood products where they can occur at up to 100 mg As/kg (wet mass). The major arsenic compound in most marine animals is arsenobetaine [$(CH_3)_3As^+CH_2COO^-$]; various studies have shown that arsenobetaine is rapidly excreted unchanged in urine and it is considered totally harmless. The next most abundant arsenicals in seafoods are arsenic-containing carbohydrates collectively referred to as arsenosugars. Preliminary metabolic studies on these arsenicals have shown that they are transformed into a number of arsenic metabolites, most of which remain unidentified. We chemically synthesised one of the major naturally-occurring arsenosugars and a small (safe) quantity of the compound was ingested by one human volunteer. The biotransformation of the arsenosugar was investigated by quantitatively determining the arsenic species excreted in the urine over a four-day period during which time about 80% of the ingested arsenic was excreted. Five major arsenic compounds accounting for > 90% of the total urine arsenic were found, namely, dimethylarsinothioylacetate [$(CH_3)_2As(S)CH_2COOH$, 31%], dimethylarsinate [$(CH_3)_2As(O)OH$, 30%], dimethylarsinothioylethanol [$(CH_3)_2As(S)CH_2CH_2OH$, 17%], dimethylarsinoylethanol [$(CH_3)_2As(O)CH_2CH_2OH$, 7%], and an unknown arsenical (10%). In addition, traces of dimethylarsinoylacetate [$(CH_3)_2As(O)CH_2COOH$], trimethylarsine oxide, unchanged arsenosugar and its thio analogue were identified in the urine. Our study reports the first identification of dimethylarsinothioylacetate and two new thioarsenicals in human urine, and suggests that the thioarsenicals are likely to be significant urine metabolites after ingestion of some foods. Preliminary results on the cytotoxicity of these new arsenic metabolites will also be presented.

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THE NUTRITIONAL PREVENTION OF CANCER 400 MCG/DAY SELENIUM TREATMENT SUB-STUDY

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One of the most influential experimental evaluations of selenium as a cancer chemopreventive agent, the Nutritional Prevention of Cancer (NPC) trial was designed to evaluate the impact of selenium upon recurrence of nonmelanoma skin cancer in a high-risk population. The intervention, 200 mcg/day of selenium as selenized yeast, did not decrease the recurrence of nonmelanoma skin cancer; to the contrary, it increased nonmelanoma skin cancer by a statistically significant degree. On the other hand, the NPC trial documented sizeable decreases in the incidence of total cancer, lung cancer, colon cancer and prostate cancer, and total cancer mortality. A small sub-study attached to the Nutritional Prevention of Cancer trial, conducted among 400 people in one of the study sites, considered the impact of a 400 mcg/day treatment with the same selenized yeast formulation. Although the 400 mg treatment failed to decrease the recurrence of nonmelanoma skin cancer, it did not increase recurrence risk. Thus, this randomized, double-blind clinical trial of high risk people provides no evidence that modest selenium supplementation above even 200 mcg/day increases skin cancer risk.

IMPORTANCE OF DOSAGE AND EXPERIMENTAL DESIGN IN TRIALS TESTING THE EFFECT OF MAGNESIUM SUPPLEMENTATION ON HYPERTENSION

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Intervention studies with magnesium therapy for hypertensive patients have led to conflicting results. Comparing these studies' magnesium dose (Mg) and analyzing experimental design lends understanding to this seeming conflict. A Medline search was undertaken for studies testing Mg supplements' effect on blood pressure in humans. Full manuscripts were retrieved. Data were collected from each abstract for dose of Mg supplement and effect on blood pressure. Each manuscript was studied for circumstances of experimental design that might impact results. Studies' data and design were tabulated in ascending order of daily Mg supplement dose. Of eleven studies supplementing subjects with 360 mg Mg per day or less, nine showed no statistically significant effect on blood pressure. Of the two studies that did show a blood pressure drop at 360 mg per day, one was conducted on hypertensive adults with long term diuretic use, and the other showed a drop in blood pressure only when Mg supplements were taken after the placebo period in a cross-over design. In contrast, of the eight trials where subjects were given 480 mg or more of Mg daily, all did lower blood pressure significantly except for one where subjects were Mg replete at start of the trial. Of all trials, only one "titrated" the Mg dose with 3-week trials at three different daily doses of Mg for each subject; only doses 480 mg or above showed a significant drop in blood pressure. Choice of dosage, use of anti-hypertensive medications and experimental design can impact results in clinical tests of Mg supplements' effect on hypertension. Rather than a conflict, these results show that daily Mg supplementation at doses less than 480 mg per day will not significantly lower high blood pressure in untreated hypertensive patients, but doses of 480 mg or more per day of Mg will lower high blood pressure in unrepleted subjects.

PERFORMANCE AND CARCASS TRAITS OF BROILERS FED DIETS CONTAINING ORGANIC ZINC (BIOPLEX ZN®)

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Zinc has a role in immune function and epithelial tissue maintenance and repair (Downs et al. 2000). A 42-day trial was conducted to investigate the effect of organic zinc (Bioplex Zn®) on performance and carcass traits of Ross male broilers fed diets containing organic selenium (Sel-Plex®). Treatments consisted of adding increasing levels of organic zinc (T1-0, T2-15, T3-30, T4-45 and T5-60 ppm) to a corn-soybean meal basal diet supplemented with 50 ppm Zn from an inorganic source (zinc sulphate). A total of 1960 birds (392/trt) were randomly distributed into 35 pens (7 reps/trt, 14 birds/m2). Increased supplemental organic zinc to the basal diet did not influence body weight gain, feed intake, feed conversion and mortality. However, progressive addition of organic zinc increased the epithelial cell layers, the collagen content, sped up healing, and decreased skin tearing and inflammation. These results indicate that organic zinc has no effect on growth performance, but improves skin quality. The 45 ppm organic zinc addition level is required to maximize skin quality and to reduce skin tearing, which normally accounts for 5% of downgrades at processing.

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EVALUATION OF THE EFFECT OF IODINE INTERVENTION PROGRAMME ON HYPOTHYROID SCHOOLCHILDREN IN RURAL AREA OF TEHRAN

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Northern rural areas of Tehran, situated in the mountainous region, had been shown to have severe iodine deficiency in our previous studies. In 1989 the inhabitants of these villages: Kiga, Keshar, Randan, Sangan in North west and Ahar and Zagoon in North east of Tehran, received iodized oil injections followed by iodized salt distribution in 1993. The aim of this study was to evaluate the effect of iodine supplementation on iodine deficient hypothyroid school children of these villages. 571 students aged 6-14 years were studied. Goitre was graded according to the WHO classification. Serum thyroid hormones and thyroid stimulating hormone (TSH) concentration were determined using commercial kits and urinary iodine was measured by digestion method. IQ was estimated by the Raven test. The results were compared with data from our previous study in 1989. Total goiter rate decreased by 42% as compared to that in 1989. A significant decrease in grade 2 goiter concomitant with an increase in grade 1 goiter was seen, $P < 0.001$. The studied variables before and ten years after iodine supplementation were: median urinary iodine excretion 2.0 vs. 19.0 µg/dl, $P < 0.001$; T4: 6.5 ± 2.0 vs. 8.4 ± 1.6 µg/dl, $P < 0.001$, T3: 177 ± 38.0 vs. 145 ± 29.0 ng/dl, $P < 0.001$; TSH: 10.8 ± 15.1 vs. 1.8 ± 0.8 µU/ml, $P < 0.001$; IQ: 89 ± 11 vs. 95 ± 11, $P < 0.05$. No correlation was found between thyroid hormones and TSH on the one hand, and goiter, IQ and urinary iodine, on the other hand. Serum T4, T3 and TSH concentrations were within the normal range in all school children in this study. This study shows that euthyroidism induced by administration of iodized oil in hypothyroid iodine deficient schoolchildren is sustained following iodized salt consumption.

EFFECTS OF CONSUMING FISH ON IRON BIOAVAILABILITY IN IRON DEFICIENT WOMEN WITH THE G277S TRANSFERRIN GENE MUTATION

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Iron deficiency anaemia is the most common nutritional deficiency in the world, being more prevalent in women of childbearing age and children. Genetic variants in the iron regulatory systems influence the likelihood of iron deficiency. The G277S transferrin mutation is associated with higher risk of iron deficiency in menstruating women, but the mechanism is not clear because the biological activity of transferrin is not perturbed by the mutation. Non-haem iron absorption is modulated by dietary constituents, including fish which has been reported to enhance non-haem iron absorption. We examined the effect of sous vide processed fish on non-haem iron absorption from vegetables in a group of non-anaemic (Hb > 110g/L) menstruating women with low iron stores (ferritin < 20 μg/l), either with the G277S/G277G genotype (n = 10) the transferrin gene or wild type G277G/G277G (n = 15). Iron absorption was measured using stable isotopes and the erythrocyte incorporation technique. Results will be presented on the effects of both the mutation and fish intake on iron absorption.

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METAL CHELATES OF 2-HYDROXY-4-METHYLTHIOBUTANOIC ACID FOR HUMAN AND ANIMAL NUTRITION: PRELIMINARY CHARACTERIZATION OF ZINC CHELATE (ZNMHA) BY IN VITRO STABILITY AND BIOAVAILABILITY STUDIES

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The α-hydroxyacid-2-hyroxy-4-methylthiobutanoic acid (the so-called methionine hydroxyanalogue, MHA), largely used in animal nutrition as a source of methionine, forms stable chelates with divalent metals of formula $[\{CH_3SCH_2CH_2CH(OH)COO\}_2M]\cdot nH_2O$. Protonation and zinc(II) complex (ZnMHA) formation constants have been determined by pH-metry at 25°C. Distribution diagrams have shown that a zinc chelate is present even in acidic solution (pH 4.5). ESI-MS (Electron-Spray Ionisation Mass Spectrometry) investigations carried out on ZnMHA solutions confirmed the presence of chelate species, suggesting that the ZnMHA could be stable at the low acidic pH of the first digestive tract. To test intestinal bioavilability of ZnMHA, the human intestinal cell line Caco-2 was used. Toxicity studies were performed treating differentiated cells grown on filter with ZnMHA and $ZnSO_4$ (200-400 μM) for 2 h from the apical chamber and measuring variation in trans-epithelial electrical resistance (TEER). No toxic effects were observed up to 300 μM within the first hour, whereas a reduction in TEER, indicating a damage to the cell monolayer, was observed from 1 to 2 h for both Zn compounds.

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ASSESSMENT OF PLASMA ZINC IN CIRRHOTICS CHILDREN AND ADOLESCENTS

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Nutrition deficiences are common in patients with chronic liver diseases and they are at risk of zinc deficiency (Loguercio et al 2001). The aim of this study was to assess plasma zinc (Znp) concentration in cirrhotics children and adolescents and relate their nutritional status to their degree of liver impairment. Subjects (n = 57) aged 4–216 months, were distributed in 2 groups: I - 31 cirrhotics, followed at the Pediatric Gastroenterology Service at Hospital de Clínicas de Porto Alegre, and II - 26 well nourished controls without liver disease. According to Child-Pugh classification, 16 ppatients were in stage A and 15 were B + C. The study included 10 patients with biliary atresia, 9 with autoimmune disease, 2 other causes and 10 criptogenic. Dietary intakes were recorded (3 days) and anthropometric indices: height/age and weight/age Z scores, triceps skinfold thickness and upper arm circumference. Znp determinations were carried out by atomic absorption espectrophotometry, deficiency values < 70 mcg/dL. The study was approved by the hospital Ethical Committee. Results indicate there was no relationship between nutritional status, sex, age and Znp. Dietary zinc intakes were adjusted to 21/28 cirrhotics and for all controls. Znp concentrations were normal in control group (103,44 ± 18,56) but deficiency was determined in 13/31 (42%) cirrhotics, particularly with cholestasis (59,14 ± 17,29; p = 0,001). Patients Child-Pugh A had Znp mean concentration of 90,34 ± 22,41 and B + C of 59,63 ± 14,85 mcg/dl (p = 0,0001). The prevalence of hipozincemic cirrhotics children and adolescent was 42%, and it is related to liver disease impairment according to Child-Pugh criteria.

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BIOAVAILABILITY OF ZINC GLYCINATE IN COMPARISON TO ZINC SULFATE IN PHYTATE CONTAINING DIETS

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The objective of this study was to quantify the bioavailability of zinc (Zn) from sulfate and glycinate as representatives of inorganic and organic zinc sources. A semi-synthetic basal diet (native Zn: 2 µg/g) was fortified with pure sodium-phytate (8 g/kg) and with either 53 µg/g of Zn from sulfate (Control) or with 10 µg/g of Zn from either sulfate (ZnSulfate) or glycinate (ZnGly). Twenty-four 65Zn-labeled, growing rats were allotted to the three diets and were kept pair-fed to ZnSulfate for 15 d. Zn contents in blood plasma, femur and whole body, as well as, plasma alkaline phosphatase activities were reduced compared to Control indicating a zinc deficiency in ZnSulfate and ZnGly treatment. True absorption of dietary Zn was significantly higher in ZnGly than in ZnSulfate while losses of endogenous fecal Zn and urinary Zn were not affected to a quantitatively relevant extent. This resulted in a significantly improved Zn retention for ZnGly (+30%) and a lower severity on Zn deficiency symptoms compared to ZnSulfate. Metabolic utilization accounted for 95% of absorbed dietary Zn for both Zn sources. Overall, the bioavailability of zinc glycinate was significantly superior by 16% to zinc sulfate, mainly due to a higher absorptive potential at presence of a strong anti-nutritive component (phytate) in the diet.

ALIMENTARY PROVISION OF MAJOR AND TRACE ELEMENTS IN MUSCOVITES

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An estimation of chemical elements alimentary intake in Moscow residents was carried out. For this study Ca, Mg, Cu, Zn, Fe, Mn, Cr, Se, Pb, As, Ni and Co content in main food products was determined using ICP-AES and ICP-MS methods. All foods were purchased in Moscow supermarkets. The data obtained was used for calculation of the micronutrients intake from average and factual rations of Muscovites (n = 96). It was found that average daily intake of calcium in women was below the adequate level (1250 mg/day, or 52%) accepted in the Russian Federation. Supply of rations with the other chemical elements corresponded to the recommended values. It was noteworthy that zinc and magnesium intake from factual rations in women was at the lower limit of adequate range (12 mg/day and 400 mg/day, respectively). Occurrence of chemical element deficiencies (below adequate level) and excesses in women's rations was also studied. Excess of lead, cadmium, arsenic and nickel in daily rations was calculated according to literature data of average daily consumption (Anke 2004). The occurrence of lead excess in women's factual daily rations was found to be 2% while that of nickel was 18% of the total observed persons. Occurrence of alimentary deficiencies in women was estimated as 91% for calcium, 35% for magnesium, 34% for iron, and 25% for zinc. The copper, manganese, selenium and chromium deficiencies in factual rations were minimal (< 5%).

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ZENITH PROJECT: SALIVARY CORTISOL AND MOOD IN OLDER ADULTS, MEDIATING EFFECTS OF ZINC

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Objective: To investigate the effects of zinc supplementation on cortisol levels and mood in healthy older adults. Rationale: Cortisol effects mood and behaviour as it alters brain cells and some neurotransmitters. Negative mood has been associated with increased cortisol levels (Gold et al.1986), positive mood is associated with lowered cortisol levels (Rudolph & McAuley, 1995). The latter finding is less conclusive and more research is needed (Hubert & de Jong-Meyer, 1991). Zinc supplementation may enhance positive mood (Fabris & Macchegiani, 1995) by its effect on tryptophan, which increases levels of serotonin leading to a reduction in cortisol levels (Hambridge & Mills, 1989) and an inhibitory effect on negative affect. Little research has been carried out looking at this in older adults. Methods: This is a randomised placebo controlled double blind intervention study investigating supplementation of either 15 or 30 mg Zn/day or placebo for six months on mood and cortisol levels. A Northern Ireland sample of 29 older adults, aged 55-70 years was recruited. Baseline and 6 month follow up measures of salivary cortisol, measured using an enzyme immunoassay kit, were obtained twice a day for 7 consecutive days in conjunction with measures of positive and negative affect measured using the PANAS scale (Watson et al. 1988). Results: Data analysis was performed using SPSS employing multivariate procedures with repeated measures. Conclusions: Zinc supplementation, mood and cortisol levels in older adults will be discussed.

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ZENITH PROJECT: ZINC SUPPLEMENTATION AND COGNITIVE FUNCTION IN AGEING EUROPEANS

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Objective: To determine if zinc supplementation improves cognitive function in older adults. Rationale: Cognition declines with age (McDaniel et al. 2002), and may be associated with an age related decline in serum zinc levels (Kretsch et al. 1999). Zinc is important for development and function in the brain (Sazawal et al. 1996) and is found in areas responsible for learning and memory (Crawford, 1983). Zinc supplementation may be beneficial to cognitive functioning in older adults (Ortega et al. 1997). Methods: This is a randomised placebo controlled double blind intervention study investigating supplementation of either 15 or 30 mg Zn/day or placebo for six months on cognition. It is a European multicentre study, with centres in France, Italy and Northern Ireland. A total of 387 community dwelling volunteers were recruited, aged 55-87 years old with equal numbers of males and females. A computerised neuropsychological test battery (CANTAB) (Robbins et al. 1994) was used to assess cognition, at baseline, 3 and 6 months of follow up. Results: SPSS was used to analyse data, employing multivariate procedures. Conclusions: The effects of zinc supplementation on various aspects of cognition in older adults will be discussed.

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ZINC INTAKES IN TRADITIONAL COMPLEMENTARY FOODS EATEN IN THE NORTH OF CAMEROON

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Complementary foods chosen after interviews near mothers with babies under 36 months, living in the North province of Cameroon were studied to determine zinc level and intakes. The foods were selected for their availability and frequent consumption, cooked in triplicate for reproducibility, kept at -20°C or analysed immediately. The quantities of food eaten were measured by the difference of weight at the beginning and the end of children feeding using double dishes method. Total zinc levels obtained by atomic absorption ranged between 0.66 ± 0.05 mg/100g dry weight in sweet potatoes and 2.57 ± 0.24 mg/100g dry weight in Irish potatoes with fish. Zinc contents in analysed meals were very low when traditionally cooked. There were no best sources of zinc. Zinc intakes estimated from quantities of food consumed and zinc content in meals ranged between 0.15 ± 0.04 mg/day in sweet potatoes and 2.41 ± 0.27 mg/day in Irish potatoes with fish. The greatest combinations in three

meals could cover only 10% to 49% of recommended daily zinc intakes below 6 months and between 7 and 12 months respectively. These low zinc levels in complementary foods in absence of zinc supplementation may lead to zinc deficiency, which is still not well addressed as public health problem in Cameroon.

IONIC MANGANESE SUPPLEMENTATION IS BENEFICIAL TO CAENORHABDITIS ELEGANS

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We used Caenorhabditis elegans, a simple multi-cellular organism, to investigate the role played by ionic manganese as there are several reports supporting manganese as a radical scavenger in unicellular organisms. Manganese also has a dark side as it causes Parkinson's disease-like syndrome and DNA fragmentation. Unlike iron and copper we know very little about the cellular roles of this metal ion and the goal of this study is to uncover if manganese has any free radical scavenging properties in a higher organism. In our study we found that with manganese supplementation the mean life-span of an oxidatively challenged short-lived C. elegans (mev-1) was significantly increased. We also found that at the levels we were using manganese did not appear to be toxic. Manganese supplementation also did not alter the development or the fertility of the worms negatively. In fact supplementation at a higher level (0.5-1 mM) accelerated the development and increased the total fertility of the wild-type worms by 10%. Internal manganese levels were measured in manganese supplemented worms using ICP-MS (inductively coupled plasma mass spectrometer) and the data obtained suggests that manganese supplemented to the growth medium is taken up by the worms. Although manganese appears to be beneficial to the worms, it is unclear whether it is working directly as a free radical scavenger or indirectly by up regulating several house keeping antioxidant enzymes in C. elegans.

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PRENATAL ZINC TREATMENT AT THE TIME OF ACUTE ETHANOL EXPOSURE LIMITS SPATIAL MEMORY IMPAIRMENTS IN MOUSE OFFSPRING

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Prenatal subcutaneous Zn treatment at the time of acute ethanol exposure has previously been demonstrated to protect against physical birth abnormalities in mice. The current study examined whether Zn treatment can also prevent the more subtle cognitive impairments caused by ethanol exposure in early pregnancy. Pregnant C57BL/6J dams were injected with saline (0.85%) or 25% ethanol (0.015 ml/g) intraperitoneally at 0 and 4 h on gestational day (GD) 8. $ZnSO_4$ treatment was administered subcutaneously (2.5 µg Zn/g at 0 h) immediately following ethanol treatment. Offspring of normal physical appearance were randomly selected from litters for each of the three treatment groups and were tested at 55 days of age using a crossmaze-water escape task that tests for spatial learning and memory impairments. No differences were observed between treatments for the spatial learning task. However, young adult mice exposed to ethanol in utero demonstrated impaired spatial memory ($p < 0.05$), with a decrease in correct trials and increased escape latency and incorrect entry measurements, compared with saline-treated controls. In comparison, offspring given subcutaneous Zn treatment at the time of ethanol exposure were not cognitively impaired, performing the same as control mice in the cross-maze escape task. These findings indicate that critically timed Zn administration can limit ethanol-induced spatial memory impairments

SELENIUM-ENRICHED EGGS IMPROVES SELENIUM STATUS IN HUMAN VOLUNTEERS

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Selenium deficiency is widespread across the globe. The aim of the present work was to evaluate Se-enriched eggs as a source of Se for human consumption. Student volunteers were stratified by age and sex and then randomly allocated to groups designated to consume either two designer or two commercial table eggs per day for eight weeks in a double-blind trial. Sixty volunteers (30 in control and 30 in experimental group) successfully finished the trial. Eggs consumed in the control group contained 7-9 µg Se/egg and experimental eggs: 28-32 µg Se/egg. Blood was collected before the beginning and at the end of experimental period and Se was determined in plasma by hydride generation atomic absorption spectrometry with fluorometric detection. Statistical analysis was performed using ANOVA. The level of selenium in plasma of volunteers living in the Kiev area of Ukraine (0.055-0.081 µg /ml) was on the low side of the physiological range and was somehow lower than we reported earlier in volunteers in Scotland. Consumption of commercially available eggs only slightly increased Se in plasma, which reached physiological level (0.075-0.085 µg/ml). In contrast, consumption of Se-enriched eggs for eight weeks was associated with a significant increase in Se concentration in plasma. Plasma Se reached 0.09- 0.14 µg/ml, indicating improving Se status of volunteers.

SELENIUM STATUS OF NEW ZEALAND CHILDREN: NATIONAL CHILDREN'S NUTRITION SURVEY 2002

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The aim of this study was to assess the selenium status from serum selenium concentrations and selenium intake in the first 2002 Children's Nutrition Survey in New Zealand. A nationally representative sample of 3275 children (response rate 69%) aged 5-14 years (representing Mäori, Pacific, and New Zealand European and other (NZEO) ethnicity) was recruited and stored serum was available from 1547 children (34% eligible children) for analysis of selenium. Selenium intake was determined from 24 h diet records and analysed using the NZ Food Composition Database. The mean (SEM) serum selenium concentration was 0.97 (0.02) µmol/l; males (0.99 (0.01)) had higher serum selenium than female children (0.95 (0.01); p = 0.000). Mäori children had significantly lower serum selenium (0.94 (0.01) µmol/l) than Pacific Island and NZEO children (0.97 (0.01) µmol/l, p = 0.005, p = 0.003, respectively). As a whole, our children fall in the middle of the range of international serum selenium concentrations. However, clear regional differences from North to South were observed. Children in Auckland/Northland (1.07 (0.02) µmol/l) had mean serum selenium levels higher than all other regions (p = 0.000, p = 0.43), while Otago/Southland (0.82 (0.03) µmol/l) and Canterbury/Marlborough (0.77 (0.02) µmol/l) had the lowest. Mean selenium intake of these children was 35.8 (1.3) µg/d (males 40.6 (2.2); females 30.9 (1.3) µg/d). A reference range for serum selenium concentrations for New Zealand children aged 5 to 14 years of 0.673 to 1.319 µmol/l (53 to 104 µg/l) was established from the 2.5 to 97.5 percentiles.

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METABOLIC AND GROWTH RESPONSES TO DIETARY COBALT IN FATTENING STEERS FED CORN OR BARLEY-BASED DIETS

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Sixty steers were used to determine the effects of dietary cobalt (Co) on performance, plasma, liver and ruminal metabolites of cattle fed corn or barley-based diets. The design was a 2 × 3 factorial with factors being corn or barley-based diet, and supplemental Co at 0, 0.05 or 0.15 mg/kg diet DM. The non-Co-supplemented corn and barley control diets contained 0.04 and 0.02 mg Co/kg DM, respectively. Cobalt supplementation improved gain and feed intake, increased plasma and liver vitamin B12 and folate, decreased plasma methylmalonic acid (MMA), decreased plasma and ruminal succinate, increased ruminal molar proportion of propionate, and decreased ruminal proportions of acetate and butyrate, regardless of grain source. Increasing supplemental Co from 0.05 to 0.15 mg/kg diet did not significantly affect liver or plasma vitamin B12, but decreased plasma MMA and succinate and ruminal succinate concentrations. Ruminal fluid vitamin B12 concentrations on day 84 were increased by dietary Co in steers fed corn, but not barley-based diets. Metabolic characteristics affected by Co deficiency were altered to a greater degree in steers fed barley vs corn-based diets. The highest level (0.15 mg) of supplemental Co evaluated eliminated differences among barley and corn-diets in some metabolites (liver folate, ruminal butyrate) altered by Co deficiency, but not others (plasma vitamin B12, plasma and ruminal succinate). Results suggest that ruminal synthesis of vitamin B12 differs among cattle fed barley vs corn diets.

THE SEARCH FOR CLUES TO IMPROVE PROSTATE CANCER THERAPIES; SELECTION OF DIETARY TRACE ELEMENTS AND OTHER NUTRITIONAL COMPONENTS

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Carcinoma of the prostate (CaP) is still today potentially an incurable age-linked disease of unknown aetiology. The low incidence of CaP in Japan and Italy (compared to the > tenfold in Northern Europe and USA), may be linked to the intake of soy and parmesan respectively, which both contain Serine amino acid, further supported by the lycopene intake from tomatoes. Dietary correction of the aetiological metabolic deficiency, leading to stimulation of adrenal zona reticularis cells seem to have a central position . Detection of an elevated prostate specific antigen (PSA)-level should always lead to further assays for follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), dehydroepiandrosterone (DHEA), dehydroepiandrostendione-sulphate (DHEA-S), steroid hormone binding globuline (SHBG), and s-Ferritin to access prognosis and changes for dietary medical treatment modalities. The suggestion for treatment of CaP patients: 1) Oral administration of each (2-5 g/d) of respectively L-amino acids: Arg, Asp, Glu, Gly, Lys & Ser. 2) Trace elements (1-3mg/d); CrCl 2.6H20 6 mg (Cr 1.17 mg); SnCl 4.5H20 4 mg (Sn 1.35 mg); SrCl 2 1-7 mg (Sr 2 mg); Na 2 VO5 4H20 6 mg (V 2.5 mg); Na2 WO4 2H20 4 mg (W 2.3 mg). Strontium is applied to patients with bone metastases who have been orchiectomized while the oncogen transcription has been forced to obey normal transcription by a mitrochondrial regulatory function. 3) Small physiologic amounts of vitamins A,B,C,D,E,K, folic acid (2 mg/d), and Lycopene. 4) To improve lymphopoiesis and the immunodefence of patients a diet containing prion-free Neurogenic lipids (Neurofood Ltd, Finland) has been recommended. Over 30 years of clinical and experimental studies with this treatment modality evolved to a strong belief that malignant gene-transcription can be corrected, and genetic weakness can be compensated for, by natural bio-modulating dietary means.

THE EFFECTS OF DIETARY MOLYBDENUM, SULPHUR AND IRON ON THE PITUITARY GLAND TRACE ELEMENT CONTENT AND FUNCTION

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Administration of ammonium tetrathiomolybdate (TTM) has been shown to result in changes in the pathology of the pituitary gland in sheep, with a marked depletion of hormones and altered trace element content (Haywood et al. 2004). Parenteral administration of TTM has been recommended as a treatment for copper toxicity. However, TTM is also produced by rumen microbes from ingestion of dietary molybdenum and sulphur. The aim of this study was to investigate the effects of dietary Mo on pituitary function in lambs. Fifty female lambs were fed varying levels of molybdenum (2, 5 and 10 mg/kg DM) along with sulphur (2 g/kg DM) or iron (500 mg/kg DM) and sulphur (2 g/kg DM) for a period of thirteen weeks. Copper levels were 6.1 mg/kg DM. After the end of the trial the lambs were killed and their pituitary glands and livers were removed for analysis. Trace element content was determined by ICP-MS and pituitary function was assessed by immunocytochemistry labelling for adrenocorticotrophic hormone (ACTH). Plasma Cu was significantly elevated in the lambs receiving 10 mg/kg Mo compared with all the other treatments. Liver Cu levels were significantly higher in control lambs and liver Mo significantly increased with Mo supplementation. There were no effects on pituitary Cu or Mo levels. However, immunocytochemical labelling showed a marked accumulation of ACTH in response to dietary Mo. In conclusion, dietary Mo caused alterations in Cu metabolism in lambs and interfered in the release of the pituitary hormones, possibly via alteration in the activity of peptidylglycine α-amidating monooxygenase.

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SHORT TERM TISSUE ZN EXCHANGE IN 65ZN LABELLED ADULT RATS

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The quantitative contribution of tissues to metabolism of absorbed dietary Zn was investigated in a model study with 32 adult rats. Animals were trained to consume a restricted amount of feed with sufficient Zn content in one portion per day. ^{65}Zn was added to one portion and animals were sacrificed at 0, 1, 2, 4, 8, 24, 28 and 32 h past feeding. Animals were dissected quantitatively in various tissues, gut contents and excrements collected past tracer feeding . About half of labelled Zn was absorbed. Liver quickly increased Zn content up to 8 h past feeding (maximum: 20% of absorbed Zn) and then lost Zn, which demonstrates the significance of this organ to serve as a short-term buffer of absorbed Zn. More slowly, the dominant proportion of absorbed Zn was transferred to muscle and fat tissue (50%) and the skeleton (25%). This confirms former estimates on the size of mobile Zn pool in these tissues and the significance of the skeleton to serve as a quantitative Zn storage. Radiography revealed the newly incorporated bone Zn to be distributed among the entire bone tissue. There was no indication of a specific contribution of osteocytes to incorporation of bone Zn.

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A SINGLE DOSE PHARMACOKINETIC AND PHARMACODYNAMIC COMPARISON OF TWO CALCIUM SUPPLEMENTS IN PRE-MENOPAUSAL WOMEN

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It is well documented that parathyroid hormone (PTH) governs the release of calcium from bones. It could be hypothesised that suppression of PTH in a consistent way prevents bone loss and osteoporosis. A study was undertaken to evaluate the pharmacokinetic and pharmacodynamic characteristics of Aquamin F, Calcium Carbonate and Placebo in women. In this randomized, double blind, placebo-controlled crossover trial, patients (n = 12) were given a single oral dose of each treatment. Bloods taken throughout the trial were assessed for ionized and total calcium, magnesium, phosphorus and PTH. A 12-h urine collection was conducted. There was no difference between the treatments in regard to ionized and total calcium levels. Aquamin F revealed a significantly ($p = 0.004$) greater urinary clearance of calcium than the Calcium Carbonate as compared to placebo. There was no difference in the PTH area-over-the-curve measurements between the Aquamin F and Calcium Carbonate treatments but the Aquamin F demonstrated a more prolonged suppression of PTH levels at 120 and 240 min ($p = 0.02$ and $p = 0.03$, respectively). There were no significant differences in any of the other measured variables. Aquamin F was found to be more bioavailable than Calcium Carbonate when compared to placebo as demonstrated by a greater calciuric response. Aquamin F resulted in a more prolonged decline in serum PTH levels.

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RAPID AND ROUTINE BIOMEDICAL TRACE ELEMENT ANALYSIS USING ICP-MS

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Elemental analysis in biomedical samples is currently achieved using mainly flame and graphite furnace atomic absorption spectroscopy. Flame AAS although effective for measuring Na, K and Zn in serum and urine is not sufficiently sensitive for determining Se in serum and Pb and Cd in whole blood. For this reason GFAAS, with its higher sensitivity, has become accepted as the benchmark technique for trace elemental biomedical analysis. However, GFAAS has its limitations. It is relatively slow, prone to contamination and can suffer from relatively poor precision compared to ICP-MS, in some assays. Despite the multi-element, low detection limit, excellent precision and high sample throughput capabilities of ICP-MS, this technique has not yet been widely adopted by the biomedical community, arguably because of the perceptions that it is prone to interferences, complex to use and expensive. Interference problems and complexity have been significantly reduced in recent years, through the development of collision cell interference removal technology and simplification of the software and hardware. From an expense perspective, the cost per sample of ICP-MS compared to GFAAS is highly dependent on the sample workload and the number of elements required to be measured. At high sample throughput (more than 20 samples per h) and for multi-element assays, ICP-MS soon becomes more cost-effective than GFAAS. ICP-MS has the additional benefit over GFAAS in that it can be easily interfaced with liquid or gas chromatographic separation systems to facilitate sensitive, rapid and accurate elemental speciation measurements, thereby increasing its value to the biomedical community. This paper will discuss advances in routine elemental analysis in biomedical samples using ICP-MS and will briefly discuss its potential for emerging biomedical assays, such as speciation.

ZINC STATUS AND COGNITIVE FUNCTION OF PREGNANT WOMEN IN SOUTHERN ETHIOPIA

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Effects of zinc (Zn) deficiency on cognitive function during the reproductive cycle have not yet been delineated. From three rural communities in the Siddama area of the Southern Nations and Nationalities People's Region (SNNPR) of Ethiopia, 105 women >24 weeks of gestation volunteered to participate in this study. Mean height-for-age Z score was -1.4 based on the NCHS/WHO reference data. Most women had never attended school. Dietary staples were unrefined maize and an indigenous tuber, enset. Less than 15% had consumed meat or fish during the previous three months and more than 98% reported being food insecure often or sometimes. Mean (± SEM) plasma Zn concentration was 46.2 ± 0.8 µg/dL [well below the 2.5 percentile]. Mean hemoglobin was 11.96 ± g/dL, hematocrit was 34.5 ± 0.34% and mean corpuscular volume was 89.0 ± 0.4 fL. The Raven's Colored Progressive Matrices (CPM) were administered individually to each woman. Summed scores for scales A, Ab, and B ranged from 5 to 23. Plasma zinc predicted 7% of the variation in Raven's CPM scale A scores and the highest plasma zinc quartile had significantly higher Raven's CPM scale A scores than the lower two quartiles. It is concluded that pregnant women in this population are markedly zinc-deficient and that this deficiency is associated with impaired cognition.

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ELEMENTAL STATUS OF CHILDREN AND ADULTS FROM NORTH-EASTERN SIBERIA

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Elemental status of 250 Evenks (100 children and 150 adults) residing in the northernmost district of Yakutia (North-Eastern Siberia), a region of climatic extremes with complicated economic and social conditions, was studied using hair elemental analysis by ICP-AES, ICP-MS methods. The reference values of elements concentration in hair were acceptable according to the data suggested by Skalny (Skalny, 2002). The study revealed a high risk of hypoelementoses in children (75% for Mg, 57% for Zn) and excess accumulation of Cr, Fe (71%), Mn, Na (85%), Pb (85%) and Cd (28%) in their organisms. This can be a cause of high morbidity rates and worsening of growth and development indices typical for children of this region. Adult population of the region was found to be characterized by increased concentrations of Fe (52%), Mn (48%) and Cr (47%) in hair, and relatively decreased concentration of Se (25% of the examined persons). A considerable number of the adults were found to have increased concentrations of toxic metals – Pb (26%) and Al (14%).

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EFFECT OF ZINC SUPPLEMENTATION ON AGE-RELATED OXIDATIVE STRESS IN EUROPEAN POPULATION : THE ZENITH STUDY

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Oxidative stress has been reported to increase with aging in relation to an increased production of free radicals and/or a decreased level of antioxidant defences. In aging, uncontrolled oxidative stress results in an increased risk of oxidative pathologies such as CVD. Zinc, as biological antioxidant, could act in preventing oxidative stress in the elderly. This study is a part of the European multicentre ZENITH study aiming to investigate the effects on health of a zinc supplementation in free living subjects in late middle-age (55-70 years old) and older age (>70 years old) receiving 15 mg/d or 30 mg/d zinc as gluconate or a placebo form for 6 months. In the present work, we measured the effects of the zinc supplementation on oxidative stress parameters monitored by plasma SH groups, TBAR's and total glutathione, in the two groups. Biological measurements were carried out at the entry, after 3 and 6 months. At the entry, only 5.6 % of the older subjects and 4.8 % of middle aged exhibited a biological zinc deficiency (plasma Zn < 10.7 µmol/L). Each dose of Zn supplementation significantly increased all measured Zn status parameters at 3 and 6 months. However, no significant antioxidant effect of Zn supplementation was observed in older subjects. These data underline the limits of a single antioxidant supplementation on oxidative stress parameters and demonstrate that, if Zn supplementation in older subjects might be useful in maintaining immunity, or bone density, or cognitive functions, this supplementation was not efficient enough to control oxidative stress.

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UPR OF ASTROCYTES TO ALUMINIUM IS SENSED BY IRE1B AND ITS APOPTOTIC EFFECT MAY BE DUE TO INHIBITION OF THE CHAPERONS INVOLVED IN PROTEIN FOLDING

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We have recently reported the ability of aluminium glycinate to induce apoptosis in primary cultured astrocytes with evidence of nuclear fragmentation and chromatin condensation (Aremu & Meshitsuka, 2005). However, the molecular mechanism of our morphological observation and those reported by other workers (Suarez-Fernandez et al. 1999, Guo & Liang, 2001) are yet to be elucidated. The present work therefore aimed at finding out the influence of aluminium on expression of apoptosis related proteins and genes in primary cultured astrocytes. Western blotting and/or semi-quantitative RT-PCR analysis did not reveal any significant effect of Al-glycinate or tunicamycin on Bax and Bcl2 protein/gene expressions. However, PARP, a nuclear protein known to be involved in DNA repair was cleaved in the presence of aluminium glycinate and this is an evidence of apoptosis. Pulse exposure and continued culture in normal medium for about 7d showed UPR evident by up-regulation of Ire1β. In contrast, tunicamycin up-regulated Ire1α. The differential effects of tunicamycin and aluminium on Ire1α and Ire1β may indicate differences in their UPR mechanism. Moreover, aluminium glycinate in contrast to tunicamycin seem to down-regulate many gene expressions including the ER resident molecular chaperone BiP/GRP78 and Ca^{2+} binding chaperones (calnexin and calreticulin) as well as stanniocalcin 2 and OASIS. The down-regulation or none activation of the molecular chaperones whose expressions are known to be protective by increasing protein folding, may spell doom for the adaptive response which promotes

cell survival, but favor the induction of apoptotic cell death. Over-expression of OASIS, a novel ER stress sensor peculiar to astrocytes, is known to induce the transcription of BiP/GRP78 via activation of CRE and ERSE making astrocytes resistant to ER stress (Kondo et al. 2005). Thus, none induction of OASIS in the presence of aluminium may render astrocytes susceptible to death by apoptosis.

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IRON, COPPER AND ZINC INTERACTIONS

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Iron deficiency is the single most common nutritional disorder and co-exists with other micromineral deficiencies, especially with zinc deficiency. There is scarce information about the optimal molar ratios that minimize negative metals interactions. We tested the hypothesis that a combination of Fe, Cu and Zn can alter individual metal bioavailability in vitro. Caco-2 cells were cultured with different Fe, Cu or Zn concentrations in bicameral inserts. We studied ^{55}Fe or ^{64}Cu uptake and the effect of increased molar ratios between ^{55}Fe:Cu; ^{55}Fe:Zn; ^{64}Cu:Fe; ^{64}Cu:Zn; ^{55}Fe:Cu:Zn or ^{64}Cu:Fe:Zn. Metal bioavailability was studied measuring mRNA expression of Fn, MT and SCC by RT-PCR. Cells grown with increasing Fe^{+2} concentrations, showed a decreased in intracellular concentrations of Cu or Zn. Also, an increase in Cu^{+1} concentrations, showed a decrease in intracellular Fe or Zn concentrations. An increase in Fe^{+2} concentrations in the media inhibited Cu^{+1} transport, however an increase in Zn concentrations does not inhibit Cu uptake. Also, an increase in Cu^{+1} or Zn^{+2} concentrations in the media inhibited Fe^{+2} transport. The best bioavailable molar the ratio of Fe:Cu:Zn was 1:1:1. A combination of Fe, Cu and Zn have a negative effect on metal bioavailability, because they probably share similar absorptive pathways (DMT1) and physicochemical properties.

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THE EFFECT OF MICRONUTRIENTS SUPPLEMENTATION ON BACTERIAL GROWTH IN THE PRESENCE OF T-2 TOXIN

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Exposure to toxic synthetic and natural chemicals of dietary origin raise the concerns about increasing human health risks. Mycotoxins are known to produce membrane damage through increased lipid peroxidation. Antioxidant micronutrients may serve in modulating immune and inflammatory responses. They may also reduce the toxicity of several toxins on normal cells. Objective. The involvement of toxic oxygen intermediates in the bacteriostatic effect of T-2 toxin was studied by producing bacterial growth curves using turbidimetry assay in the presence and absence of oxygen radical-scaven¬ging substances e.g. selenium, zinc, vitamin E, and vitamin C. The strains used in this study includes Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus, Yersinia enterocolitica, Salmonella infantis, Erysipelothrix rhusiopathiae, Lactobacillus plantarum, Lactobacillus casei. Antimicrobial activity was evaluated as a delay of the growth curve, using a fully automated microtubidimetric method. A combination of these antioxidants provided better protection against oxygen toxicity caused by T-2 toxin for bacterial growth than they did individually. The data obtained from this study suggest that bacterial growth curve can be inhibited by T-2 toxin and that this effect can be partially counteracted by an micronutrients antioxidants such as selenium, zinc, vitamin E and vitamin C. Oxygen radicals are likely involved in the killing of bacteria and there is endogenous formation of toxic oxygen products produced by the toxin. Micronutrients and antioxidants supplementation as a means for antibacterial activity sound attractive. However, the pathophysiology of oxidative damage and the complex network of antioxidant defense systems against toxicity deserves further research.

COPPER DEFICIENCY ALTERS GENE EXPRESSION OF PROTEINS INVOLVED IN IRON METABOLISM

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Iron and copper metabolism are closely linked as they share proteins involved in their metabolism. Indeed, it is well known that copper deficiency provokes iron metabolism disorder leading to an anaemia and liver iron accumulation. Previous studies have shown that red blood cell's life is decreased during copper deficiency due to a decrease in Cu-Zn SOD activity. However, other copper dependant proteins (ceruloplasmin and hephaestin) are involved in iron metabolism. Thus, recycling disorders of iron in addition to the increased degradation of red blood cells could be involved in the disorders of iron metabolism caused by copper deficiency. The aim of the present work was to understand the interaction between copper status and iron metabolism. For this purpose we have established a dietary copper deficiency in C57BL6 male mice during twelve weeks. Haematological parameters, copper and iron status were evaluated. cDNA microarray studies were performed to investigate gene expression profiling of proteins involved in iron metabolism in the liver, duodenum and spleen. Results showed that copper deficiency induces a microcytic and hypochromic anaemia and liver iron overload. Gene expression profiling indicates that major modification of gene expression occurred in spleen. Increase in mRNA levels of transferin receptor 1 and 2 and of several proteins involved in the haem biosynthesis pathway was observed in the spleen of copper-deficient animals. These results suggest that the copper-deficient mice respond to the deficiency induced anaemia by an adaptation leading to an increase in erythrocyte synthesis.

HAS THE ROLE OF PHYTATE BEEN OVERESTIMATED IN THE ETIOLOGY OF ZINC DEFICIENCY IN CHILDREN IN NE THAILAND?

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In a survey of 567 children (age 6-13 years) from 10 rural NE Thai schools in Ubonratchathani province, 57% were zinc deficient, based on low serum zinc concentrations and age- and gender-specific IZnICG cutoffs. Diets of these children were plant-based with low intakes of animal-source foods. Phytic acid (Phy) is found in high concentrations in certain plant-based foods (e.g., cereals and legumes) and is a potent inhibitor of zinc absorption. Hence, phytic acid may play a role in the etiology of suboptimal zinc status in these children. Calcium may exacerbate the inhibitory effect of phytate on Zn absoption, if intakes are high. In this study, we determined the IP5+IP6 phytate fractions (by HPLC), Zn and Ca (by AAS) of 1-d weighed duplicate diet composites from 40 of the 567 children. Accuracy and precision were established using reference materials for Zn and Ca and an inter-laboratory comparison for phytate. Mean (SD) Zn and Ca content was 5.0(1.9) and 269(38) mg/d, respectively compared to 10-15 mg and 800 mg/day for the Thai RDA. IP5+IP6 levels were unexpectedly low. Hence, the poor biochemical Zn status of these children may result from low intakes of Zn per se and not poor bioavailability caused by elevated phytate or Ca concentrations. Such low dietary Zn levels may reflect low soil Zn concentrations in the region, whereas the very low IP5+IP6 content results from the loss of water soluble phytate from glutinous rice soaked before cooking.

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CHALLENGES ASSOCIATED WITH DETERMINATION OF MANGANESE IN SERUM

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The importance of manganese in humans is recognized and it is monitored for both evaluation of possible manganese toxicity as well as for diagnosis of manganese deficiency. The main challenges in determination of manganese in serum are associated with the narrow reference range: 0.7-2.0 mcg/L, since only approximately 5% of whole blood manganese is present in serum. Preventing contamination by environmental manganese can be a challenge, and was described in multiple literature references. Sample preparation and analysis of manganese-containing biological samples should be only performed under "clean room" protocols (Cornelis et al. 1996). Even upon assuring no contamination – the problem of reporting falsely elevated manganese results (especially in serum) still can be encountered. This presentation will discuss the contamination-prevention issues and will compare several analytical approaches for the determination of manganese in urine and serum. All specimens were analyzed by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) and by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Three different ICP-MS instruments were operated with and without utilization of reaction cell or collision cell technologies. The determination of manganese in serum by quadrupole ICP-MS instrumentation resulted in elevated results as compared with the results from GFAAS or with those obtained when the reaction or collision cell technologies were applied.

The results attained for the same specimens analyzed with different analytical approaches will be compared.

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STUDIES ON THE SELENOPROTEOME IN THE CENTRAL NERVOUS SYSTEM: SELENIUM-CONTAINING PROTEINS IN DIFFERENT TYPES OF BRAIN CELLS

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Selenium (Se) seems to be involved in various metabolic processes in the central nervous system. Most of its functions are due to selenoproteins in which the element is present in the form of selenocysteine. Several selenoproteins have been detected in the human and rodent brain, but systematic studies on the cellular expression patterns of the cerebral selenoproteins are still lacking. In this study labelling with [^{75}Se]-selenite was used to obtain information on the Se-containing proteins expressed in cultures of neuronal, microglial, astroglial, oligodendroglial and cerebral endothelial cells. Cells were grown to confluence and [^{75}Se]-selenite was added. After 72 h the cells were harvested and lysed. The cell lysates were subjected to SDS-PAGE or two-dimensional IEF/SDS-PAGE to separate the proteins. The [75Se]-labelled proteins in the dried gels were made visible by autoradiography. After SDS-PAGE similar distribution patterns were found in the different types of brain cells, with about 10 Se-containing bands in the molecular mass range between 10 and 75 kDa. The bands could be further resolved in the two-dimensional separation into about 20 Se-containing spots. These patterns, too, were very similar, with the exception of two labelled spots in the 15 kDa-range that were detected in the endothelial cells in addition to the two spots in this range present in all the types of brain cells investigated.

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STUDIES ON THE SELENOPROTEOME IN THE CENTRAL NERVOUS SYSTEM: DISTRIBUTION OF SELENIUM AND SELENIUM-CONTAINING PROTEINS IN DIFFERENT REGIONS OF THE RAT BRAIN

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Several investigations have shown the importance of selenium (Se) for the central nervous system. In periods of insufficient Se intake the brain is the tissue most preferentially supplied, but it is not yet known whether such a hierarchy also exists among the different brain regions. Neutron activation analysis of Se in samples of forebrain, hindbrain, cerebellum, hippocampus and brain stem of rats fed either a low Se diet or a Se-adequate diet showed that the element was more or less uniformly distributed within the brain and that there were no significant differences in the decreases in the Se concentrations in the deficient animals. After injection of ^{75}Se-selenite the expression of the ^{75}Se-labelled proteins in the brain was considerably delayed as compared with that in the liver and the kidney. This indicates that the metabolic pathways of Se into the selenoproteins in the brain differ from those in other tissues. The ^{75}Se-labelled proteins in the homogenates and cytosols of samples from the different brain regions were separated by SDS-PAGE or two-dimensional IEF/SDS-PAGE and determined by autoradiography. After two-dimensional electrophoresis about twenty ^{75}Se-containing spots in the molecular mass range between 10 and 75 kDa could be distinguished, the majority of which could be attributed to identified selenoproteins. The distribution patterns in the brain regions were found to be similar. The main difference was a cytosolic15 kDa-spot that was much more strongly labelled in the cerebellum. All other Se-containing spots present in the brain were also detected in samples of the liver and the kidney. It can therefore be concluded that the selenoproteome in the central nervous system is not much different from that in other tissues.

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LOCALIZATION OF TRACE ELEMENTS IN THE CENTRAL NERVOUS SYSTEM BY MICRO-SYNCHROTRON RADIATION X-RAY FLUORESCENCE

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Metals and metalloids are known to be involved in various cerebral metabolic processes. The determination of their spatial distribution within the brain in health and disease may help to identify their specific sites of action and to elucidate their biological roles. A micro-synchrotron radiation X-ray fluorescence procedure (micro-SRXRF) was developed that allows scanning of histological tissue sections with a focused X-ray beam and determination of the trace elements distributed among the different compartments by means of their characteristic X-ray emission. The analyses were carried out at the SRXRF beamline at HASYLAB by means of a capillary optical device focusing the X-ray beam with a spatial resolution of 15 μm. In this way detection limits in the femtogramme range were achieved. The procedure was applied in the investigation of the cerebral distribution of several elements in transmissible spongiform encephalopathies (TSE). Brain sections were obtained from hamsters which had been infected with the TSE strains 263K, ME7-H and BSE-H. The measurements included the determination of the distribution of phosphorus, sulfur, chlorine, potassium, calcium, iron, copper and zinc and the immunohistochemical detection of the prion protein. This protein which in its physiological form binds up to five copper ions, is altered in TSE into a pathological conformation. In this investigation a strong local enrichment in copper in a spherical form was observed in the brain samples that had not been described before.

INFLUENCE OF COPPER STATUS ON THE ACCUMULATION OF TOXIC AND ESSENTIAL METALS IN CATTLE

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Interactions between toxic and essential metals, as well as between essential metals themselves, can modulate their accumulation and toxicity. In this study correlations between copper and toxic (cadmium, lead) and essential elements (molybdenum, iron, selenium, zinc, manganese and cobalt) were evaluated in tissues (liver, kidney) of 195 calves from a region in NW Spain where there is much intensive pig farming and animals usually have high hepatic copper concentrations. Metal concentrations were determined by ICP-OES and correlations between pairs of elements determined from Spearman rank correlations. Our results indicated that the high copper exposure in calves in our study leads to a higher hepatic lead deposition as well as to a tendency to a lower cadmium accumulation in the kidney. In relation to the essential elements, the positive association between copper, molybdenum, iron and zinc indicates that the high copper exposure in our animals has not an antagonism effect on these essential elements directly related to copper status. On the contrary, the association between copper and selenium could indicate animals exposed to high copper levels are suffering an oxidative damage, or alternatively, a potential role of selenium on copper accumulation.

DETERMINATION OF SELENIUM IN BIOLOGICAL SAMPLES BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

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Selenium (Se) is an essential nutrient for man and animals. It is required by certain enzymes that protect cells from oxidative damage and maintaining an adequate Se status has been linked to decreased incidences of certain types of cancer in humans. The accurate measurement of selenium in tissues is important for studying its retention and distribution by the body. The aim of this work was to apply an assay procedure for measuring total Se in feed (Connolly et al. 2003) to the analysis of biological tissue samples. Digestion conditions were optimised to allow for complete destruction of organic Se species. Samples were digested in an open system using a strongly oxidising mixture of nitric and perchloric acids (2:1). Subsequent treatment with concentrated hydrochloric acid ensured that any Se (VI) formed during the initial digestion was converted to Se (IV) for hydride generation. Certified reference materials (bovine liver, dogfish muscle and pig kidney) were analysed and found to contain 0.73 ± 0.05, 1.43 ± 0.09 and 10.5 ± 0.02 mg/ kg Se respectively. These results agree well with the certified Se values of 0.73 ± 0.06, 1.4 ± 0.09 and 10.3 ± 0.5 mg/ kg Se and suggest that the method is suitable for measuring total Se in these types of sample.

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GENE EXPRESSION ANALYSIS OF U937 HUMAN MONOCYTE/MACROPHAGE CELLS AFTER ERYTHROPHAGOCYTOSIS USING A FOCUSED CDNA MICROARRAY

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Each day, approximately 300 billion erythrocytes are phagocytosed by reticuloendothelial macrophages in the adult human. Catabolism of ingested red cells markedly increases the level of cellular iron, which induces the expression of several genes involved in iron handling by the macrophage. To enlarge our view of

changes in gene expression after erythrophagocytosis, we used a Human Metal Transport and Homeostasis Array (SuperArray Bioscience), which contains cDNA fragments encoding 96 genes whose expression regulates and/or responds to cellular pools of iron, copper, zinc, and selenium. U937 cells in suspension were treated with PMA for 2 d to induce differentiation into adherent macrophages. Differentiated U937 cells were incubated with or without opsonized erythrocytes for 1.5 h, and harvested 4 h later. Total RNA was isolated and used to synthesize biotinylated cDNA probes for hybridization to the gene arrays. Differentially expressed genes identified by the microarrays were further quantified by using quantitative real-time PCR. Microarray analysis revealed the differential expression of genes responsive not only to iron (e.g., heme oxygenase 1), but also to zinc and selenium status. Our findings with the focused cDNA array thus provide new insights into metal homeostasis in the macrophage after red cell ingestion.

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ZINC STATUS AND BMI OF VEGETARIANS, PRACTICING YÔGA, IN THE CITY OF SAO PAULO, BRAZIL

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According to some studies, vegetarians are at risk of zinc deficiency, although may have normal or little lower body mass index (BMI), if compared to omnivores. The objective of this study was to assess zinc status and body mass index in 30 vegetarians, practicing Yôga, in the city of Sao Paulo, Brazil. We collected blood for plasma and erythrocyte zinc analysis by flameless atomic absorption spectrophotometry. Data of weight and height were obtained, and the BMI was calculated according to Quetele´s equation. The individuals were following a vegetarian diet for more than one year, and had mean age of 28.6 years (women) and 25.1 years (men). We have as preliminary results that mean zinc plasma concentration was 68.6 µgZn/dl (66.7% < 70 µgZn/dl) for women and 76.6 µgZn/dl (12,5 < 70 µgZn/d) for men. Mean erythrocyte concentration was 38,36 µgZn/gHb (55,6% < 40 µgZn/gHb) for women and 34.75 µgZn/gHb (87.5% < 40 µgZn/gHb) for men. Mean BMI was 21.48 Kg/m^2 for women and 21.9 Kg/m2 for men. The results suggest that mean erythrocyte Zn, for both men and women, and mean plasma Zn for woman were lower than reference ranges, while Zn plasma concentration for men was adequate according to these references, showing that these vegetarians are at risk for zinc deficiency, even though their BMI means, for both sexes, were adequate. We concluded that every diet that is restrictive deserves attention for deficiencies risk, especially with concern to minerals such as zinc.

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LITHIUM PROTECTS GLIAL CELLS FROM ZINC TOXICITY IN VITRO

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Recent studies present neuroprotective actions of lithium against variety of insults (Chuang et al. 2002). Under pathological conditions, such as brain trauma, extracellular concentrations of zinc can reach neurotoxic levels. The aim of the present study was to examine the action of Li on Zn toxicity in glial cells in vitro. Two glioblastoma cell lines (1231N1 and U87MG) were cultured under standard conditions, in media containing concentrations of Zn above the physiological range (10 mg/l) with and without lithium at concentrations equivalent to the serum therapeutic level (1 mg/l). After incubation for 72 h in test media, viable cell number was determined using MTS cell proliferation assay. ANOVA revealed that incubation of both 1321N1 and U87MG cells in media containing Zn resulted in a significant fall ($p \leq 0.005$) in cell proliferation compared to control medium with or without lithium. This fall was not observed when Li and Zn were both present in the medium. These results suggest a protective effect of Li on Zn toxicity in glial cell cultures which might be of particular clinical interest.

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ANTI-SECRETORY EFFECT OF ZINC IN PIGLET SMALL INTESTINE

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Dietary zinc reduces diarrhoea in weaned piglets and in undernourished children (Poulsen, 1995, Fuchs, 1998). The mechanisms behind this effect have not yet been fully established. However, Ussing chamber studies showed that high levels of dietary zinc (2500 mg/kg) to weaned piglets reduced the electrophysiological responses to secretagogues in the small intestinal epithelium (Carlson et al. 2004). New studies aimed to determine whether this antisecretory effect of zinc was due to a short-term direct effect on the epithelial function. The effect of zinc in the bathing media (either on the mucosal or serosal side) on responses to 5 different secretagogues was studied. The results showed that the responses to 5-HT (serotonin), vasoactive intestinal polypeptide and carbachol were reduced when zinc was added on the serosal side. These results indicate that zinc after it has been absorbed has a direct antisecretory effect in the small intestine.

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ZN INCREASED EXTRACELLULAR MATRIX MINERALIZATION, BUT AFFECTS LESS ON BONE-RELATED GENE EXPRESSION IN OSTEOBLASTIC MC3T3-E1 CELLS UNDER ZINC DEFICIENCY

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Zn is an essential cofactor for enzymes involved in the synthesis of various bone matrix constituents, thus it stimulates bone mineralization in vivo and in vitro. We determined whether zinc deficiency would affect extracellular matrix mineralization and bone-related gene expression. Leptin (ob gene) receptor gene expression was also measured, since leptin is thought to be a negative effector for bone formation, although this is still controversial. Osteoblastic MC3T3-E1 cells were cultured at a concentration of 0 to 15 μM $ZnCl_2$ (Zn- or Zn+) at different time intervals. Extracellular matrix mineralization was detected by staining for calcium deposits using Alizarin Red and von Kossa stains, and for alkaline phosphatase using ALP stain. Extracellular matrix mineralization, as determined by stains of Alizarin Red and von Kossa, was increased with Zn+ and alkaline phosphatase stain was also increased with Zn+ in a conc- and time-dependent manner. However, bone-related (osteocalcin, collagen type I, osteopontin, PTH receptor and RUNX2) gene expressions were not diminished under zinc deficiency, except for alkaline phosphatase, which also showed decreased staining and enzyme activity in Zn-. Neither of the leptin receptor (OB-Ra and OB-Rb) genes was detected in this cell line. Results indicated that zinc affects extracellular matrix mineralization in osteoblasts, but it has less effect on bone marker gene expression. The current work supports the positive effect of zinc on bone calcification.

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DETERMINATION OF DEPLETED URANIUM (DU) IN RATS FOLLOWING 3- OR 6-MONTH EXPOSURE TO SURGICALLY IMPLANTED DU PELLETS

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Depleted uranium (DU) is used to reinforce armour shielding and increase penetrability of military munitions. Although there is conflicting data, concern exists regarding the role of DU in the etiology of Gulf War syndrome. We examined DU accumulation in various brain regions following surgical implantation of metal pellets in rats. Male Sprague-Dawley rats were divided into five groups: non-surgical controls (NS), sham (SH, 0/20 DU pellets), low (LW, 4/20 DU pellets), medium (MD, 10/20 DU pellets) and high (HI, 20/20 DU pellets). Rats were weighed weekly as a measure of general health. No statistically significant changes in weight were observed among any of the groups in either cohort. At the conclusion of the respective studies, animals were perfused with buffer to prevent contamination of brain samples with DU from blood. Brains were removed and dissected into six regions: cerebellum, brain stem, midbrain, hippocampus, striatum and cortex, and DU were analysed with HR ICP-MS (Finnigan Element2, Thermo Electron). One-way ANOVA of data from animals treated for six months indicates that DU significantly accumulates in cortex ($p < 0.001$ for MD and HI compared to NS and SH), the midbrain ($p < 0.01$ for HI compared to NS and SH) and cerebellum ($p < 0.001$ for HI and $p < 0.01$ for MD compared to NS and SH). Our data suggest that over time DU accumulates in various brain regions, and that elevated brain-DU levels in non-perfused rats are similar to those in perfused animals, thus reflect brain levels rather than blood levels.

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ZINC SUPPLEMENTATION HAS NO EFFECT ON IN VITRO CU-INDUCED OXIDATION OF LOW DENSITY LIPOPROTEINS IN LATE MIDDLE-AGED FRENCH. THE ZENITH STUDY

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Background: The essentiality of zinc (Zn) in nutrition and health is well established and Zn has been showed to possess antioxidant properties. As inadequate dietary Zn intake has been reported in late middle-aged and elderly subjects, Zn supplementation may prevent oxidative stress and limit degenerative disease progression in these populations. However, little is known about the antioxidant effects of Zn supplementation in these populations. Objective: We undertook this study to evaluate the long-term supplementation effects of two moderate doses of Zn on in vitro Cu-induced LDL oxidation. Design: three groups of 16 healthy late middle-aged subjects from each sex participated in the study. Each group received for six months either 0 mg/d, 15 mg/d or 30 mg/d of supplemental Zn. At the beginning and at the end of the supplementation period, plasma Zn level and the in vitro LDL oxidability were determined by assessing basal conjugated diene, the rate of diene formation and the maximum of formed diene. Results: Zn supplementation increased significantly plasma Zn levels. However, Zn supplementation was without effect on the in vitro LDL oxidation parameters. Baseline diene level, lag phase and diene production were not significantly modified. Moreover, in vitro LDL oxidability was not different between men and women. Conclusion: This study showed that a long-term moderate Zn supplementation in late middle-aged subjects had no effect on the in vitro Cu-induced LDL oxidation.

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EVALUATION OF NUTRITIONAL STATUS RELATIVE TO ZINC IN UNIVERSITY STUDENTS

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The deficiency in zinc is a worldwide nutrition problem. The objective of the current study was the evaluation of the nutritional status relative to zinc of a group of university students. The typical life style of students reflects in their nutritional habits, which are commonly interfering with their nutritional status. Blood samples and urine were collected during a period of 24 h of 60 university students. The samples were analysed by atomic absorption spectrometry. Observed zinc levels in erythrocytes ranged between < 40 µg Zn/g Hb (54,38% of examined individuals), 40-44 µg Zn/g Hb (17.54%) and > 44 µg Zn/g Hb (28.07%). Relative to zinc in plasma observed levels varied between < 75 µg Zn/dL (22.80%), 75-110 µg Zn/dL (71,93%) and > 110 µg Zn/dL (5,26%). In urine zinc levels were found between < 300 µg Zn/24 h (66,67%); 300-600 µg Zn/24 h (3.51%) and > 600 µg Zn/24 h (29.82%). Even if resulting plasma zinc levels of the majority of the students are in a normal range of the nutritional status, observed zinc concentrations in erythrocytes and urine already reached the limits. Prevention and further examination of this problem are recommended.

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BORON AND MOLYBDENUM CONTENT IN INFANT FORMULAS

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There is new evidence that the content of boron (B) (Hunt, 2004) and molybdenum (Mo) (Friel, 1999) in human milk (HM) may be regulated homeostatically. Presently, the B and Mo content of infant formulas (F) is not standardized by either statute or manufacturing practice. Our analysis of 5 representative F from Western Canada indicated Mo concentrations (ug/L, mean (range)) of 15 (4.3–21.5) in F vs 5 (2-23) in HM and B concentrations of 96 (21-176) in F vs 30 (20-60) in HM. B and Mo are natural constituents of protein such that their variable concentrations among F probably reflected differences in protein sources. Because our findings indicate higher concentrations of B and Mo in F than in HM, further research is needed to determine whether or not the forms of B and Mo are of comparable bioavailability.

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THE DIETARY COPPER INTAKES IN CHINA

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Purpose: In order to obtain the dietary copper intakes in different sex-age groups in China, we conducted this dietary study to provide reliable basic data and to establish recommended dietary copper intakes in different sex-age groups. Method: Using the Chinese total dietary study method, the analytical samples were obtained in

different sex-age groups by food consumption survey, food aggregation, food sampling and preparation. The levels of dietary copper were determined by atomic absorption spectrometry. The dietary copper intakes in different sex-age groups were obtained by the food consumption data and the copper content in different dietary samples. The dietary copper nutrition status was evaluated in China in 10 different sex-age groups by using the dietary copper AI recommended by Chinese Nutrition Association. Results: The results indicate that the dietary copper intake is lower in different sex-age groups. Only a few sex-age groups in some provinces reached AI values. Dietary copper intake in 10 different sex-age groups reached to 91.2% (2-7 years), 84.2% (8-12 years), 74.7% (13-19 years male), 73.6% (13-19 years female), 85.8% (20-50 years male), 74.6% (20-50 years female), 80.6% (51-65 years male), 70.0% (51-65 years female), 74.3% (> 65 years male), and 60.9% (>65 years female) of AI respectively. Conclusion: It is the first time that dietary copper intake of an older population has been researched in the Chinese total diet study. The result shows that the copper deficiency in older people is much more serious than in younger people. The dietary copper intake in the population over 50 years of age was lower in eight provinces than 85% of AI values. In the view of the situation, it may be necessary to extend this study on the copper nutrition status in different sex-age groups in China, especially in older people.

ORGANOSELENIUM IN YEAST (SEL-PLEX®) DOES NOT PRODUCE OVERT SIGNS OF TOXICITY IN YOUNG BROILER CHICKENS

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Broiler chickens were provided diets containing selenium supplements ranging from 0 (Negative Control), 0.3, 0.6, 1.2, 5.0, 10.0 or 15.0 ppm of sodium selenite or organoselenium (Sel-Plex®) from hatching through three weeks of age. The feeding of sodium selenite at levels greater than 0.6 ppm resulted in a decrease in overall body weights, lymphoid organ relative weights, and an increase in relative weight of liver, but body weights and relative weights the lymphoid organs, and liver of Sel-Plex®-fed broilers were not different from the weights of negative controls. Sodium selenite at 10 and 15 ppm caused a severe loss of cellularity in the thymus, which was reflected in decreased numbers of CD4+ and CD8+ T cells, but Sel-Plex® treatments showed no significant decreases in CD4+ and CD8+ T cells. Cutaneous basophil hypersensitivity in response to intradermal phytohemagglutinin was less with Sel-Plex® than with sodium selenite at nominal and toxic levels of supplementation. Glutathione peroxidase and thioredoxin reductase activities were elevated by both selenium sources and by selenium levels. The results suggest that organoselenium in Sel-Plex® is a safe and nontoxic source of selenium for poultry.

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EFFECT OF DIETARY TRANSGENIC MAIZE ON ABSORPTION AND RETENTION OF TRACE ELEMENTS

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The objective of this study was to determine the effect of conventional and transgenic maize, so-called Roundup Ready (RR), with an introduced gene of glyphosate resistance (two lots from Monsanto, USA) on trace elements. The 7 days long balance experiment was performed on 30 Wistar rats (75 g), divided into three feeding groups. The tested maize lines were the only dietary source of nitrogen in the diets so that crude protein contained in the maize lines presented 10% of the dietary dry matter. Moreover diets contained: oil 8%, crude fibre 4%, mineral mixture 5%, vitamin mixture 1% and wheat starch added to achieve 100% diet. Urine and faeces were quantifiable collected daily. Iron intake, apparent absorption and retention did not differ between groups. The Zn intake per experiment and apparent absorption were significantly lower (2.87 ± 0.13 mg) in RR2 group than in other groups (3.41-3.42 mg). However Zn retention in RR2 and control groups was similar, and in both significantly lower than in RR1 group. Although similar Cu intake in all groups and the significantly lower apparent Cu absorption in RR1 group than in the others (4.25 ± 7.08% vs. 24.60-31.35%) were observed, the Cu retention did not differ between groups (-0.040-0.084 mg). It resulted from regulatory function of kidneys in Cu metabolism.

BENEFICIAL EFFECTS OF LONG-TERM TREATMENT OF PANCREATIC BETA CELLS WITH ULTRATRACE ELEMENTS VANADATE, TUNGSTATE AND MOLYBDATE

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Vanadate, tungstate and molybdate exhibit significant antihyperglycaemic effects in models of type 1 and type 2 diabetes and it is believed that these ultratrace elements derive their biological activities largely by mimicking insulin action. However, possible effects of these elements on the function of insulin-releasing pancreatic beta cells are understudied. In the present study, clonal BRIN BD11 cells were cultured for 3 days with each ultratrace element to establish doses lacking detrimental effects on viable beta cell mass. Vanadate treatment (4 μM) had no effect on cellular insulin content but improved glucose-induced insulin secretory responsiveness. However, insulin secretion mediated by protein kinase A (PKA) and protein kinase C (PKC) activation was desensitized in vanadate-treated cells. Culture with tungstate (300 μM) and molybdate (1 mM) increased cellular insulin content and enhanced basal insulin release and the responsiveness to glucose and a wide range of other secretagogues. These observations suggest significant effects of ultratrace elements on pancreatic beta cells which may contribute to their antihyperglycaemic action.

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SELENOPROTEIN W1 EXPRESSION IN HUMAN BLOOD FRACTIONS

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The dietary intake of selenium in the UK has fallen significantly over the past thirty years, and has resulted in a decrease in mean plasma selenium concentration in the UK population. Evidence suggests that marginal plasma selenium status results in impaired immune function and antioxidant defence. There are at present no well-defined biomarkers of selenium status to enable identification of groups at risk or to define levels of selenium that are associated with optimal health, including protective effects against prostate and other cancers. Cell culture and animal studies have shown that selenoprotein W is responsive to the level of exposure to selenium and thus may be a suitable biomarker. In addition, in vitro studies have indicated that selenoprotein W has an antioxidant function. Data will be presented to show SEPW1 mRNA levels (quantitative real time RT-PCR analysis) in whole blood plus erythrocyte and peripheral blood mononuclear cell fractions. Comparisons will be made to levels in tissues including, prostate, colon, testes and skeletal muscle in order to show whether SEPW1 levels in blood can be used as molecular markers indicating selenium status in target tissues.

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IRON STATUS AND DIET IN MALE C282Y HETEROZYGOTES

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The relative importance of C282Y heterozygosity, diet and lifestyle factors in determining iron status was assessed in UK men aged 40 years and over, a population with a high prevalence of the C282Y mutation of the HFE gene. Iron status (serum ferritin, transferrin saturation, soluble transferrin receptor) and pro-hepcidin concentration

were measured in fasting blood samples from 46 C282Y heterozygotes and 92 wildtype men. Lifestyle information was collected by questionnaire. Dietary intake of total, non-haem and haem iron and dietary components known to influence iron bioavailability was determined using the Meal-Based Intake Assessment Tool. Multiple regression analysis was used to determine the extent to which C282Y heterozygosity, diet and lifestyle factors predicted iron status. C282Y heterozygosity was associated with slightly higher transferrin saturation but not with serum ferritin or soluble transferrin receptor concentration. Blood loss was negatively associated with iron status and was a stronger predictor of status than haem iron intake, alcohol intake and body mass index, all of which were positively associated with serum ferritin concentration. There was no association between pro-hepcidin concentration and C282Y genotype or iron status.

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LOW LEVELS OF ORGANIC FORMS OF CU AND ZN IN PIG DIETS REDUCE THEIR EXCRETION IN FAECES, WITHOUT SACRIFICING PERFORMANCE

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In this study, we compared organic forms of Cu and Zn (Bioplex® Cu and Zn, Alltech) with inorganic forms (Cu and Zn sulphates) on Cu and Zn status, production indices and faecal excretions of Cu and Zn in pigs. Three Bioplex diets were formulated to contain Low (25 and 40 ppm), Medium (80 and 80 ppm) and High (160 and 160 ppm) levels of Cu and Zn, respectively. A fourth diet (Standard) contained equivalent levels of Cu and Zn to the High Bioplex diet. The diets were offered ad libitum to 160 Landrace x Large White female pigs from 25 to 107 kg liveweight. Daily gain was higher ($P < 0.05$) only between 25-55 kg for the Standard diet than the Bioplex diets, with no difference between Bioplex levels. However, daily gain was similar for all diets over the entire growth period. Pigs fed Low or High Bioplex or the Standard diet had a better FCR than those fed Medium Bioplex, but again only in the grower phase. Blood parameters (Hb, RBCCu) were within normal ranges, with no indication of reduced Fe status even with the High Bioplex or Standard diet. The excretion of Cu and Zn was reduced by 83 and 65% when the Low level of Bioplex was fed instead of the Standard diet. Total dietary levels of approximately 25 ppm Bioplex Cu and 40 ppm Bioplex Zn in diets for growing and finishing pigs significantly reduce the excretion of Cu and Zn in faeces with no detrimental effect on pig performance.

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MECHANISMS UNDERLYING ANAEMIA AND IRON METABOLISM DISORDERS IN TUMOUR-BEARING MICE

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Anaemia is extremely common in patients with cancer and may have many aetiologies: the clinical state of patients, treatments or the tumour itself. In the present work, we have studied the origin of the anaemia in the mouse model of carcinoma. For this purpose C57BL6 mice were inoculated with Lewis lung carcinoma cells. The tumour growth was followed during 3 weeks and mice were killed thereafter. Saline injected mice were used as controls. Haematological parameters, iron status and mRNA levels of major proteins in the liver, duodenum and spleen were measured. After 3 weeks tumour weight reached 1.66 +/- 0.11 g (n = 12). Tumour bearing mice were anaemic as shown by reduced haematocrit, number of blood erythrocytes and Hb concentration. Tumour bearing mice had also reduced plasma transferin (Tf) saturation and spleen and liver iron concentrations. The concentration of iron in the tumour was similar to this of liver. Thus, finally the iron content was greater in

tumour than in the liver. We have shown by QRT-PCR analysis an increased expression of DMT1 and Dcytb in the duodenum, reduced expression of hepcidin in the liver and an increased expression of transferin receptor (TfR)1 and TfR2 in the spleen of tumour-bearing mice as compared to controls. These results demonstrate that rapidly growing tumour leads to iron-deficiency anaemia and the consecutive adaptation by mechanisms enhancing iron absorption. In parallel, spleen expresses more TfRs to take iron for haematopoiesis. In conclusion, iron accumulation in rapidly growing tumour is sufficient to induce severe anaemia.

EFFECT OF SE-METHYLSELENOCYSTEINE SUPPLEMENTATION ON SELENOPROTEIN W LEVELS IN PROSTATE CELLS

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Selenium and its anti-cancer effects have been demonstrated to be form-specific (Ip 1998) and dose dependent (Combs 2001). Se-methylselenocysteine (SeMC) is a naturally occurring form of selenium produced in plants including garlic, onions, leeks and broccoli and studies have indicated that SeMC is the most effective anti-carcinogenic form of selenium (Ip 2000). There is evidence that selenium supplementation can reduce the risk of developing prostate cancer, potentially through incorporation into selenoproteins, plus by additional as yet unidentified mechanisms of action. We have used microarray analysis to probe the long-term effects of SeMC dose on cultured human prostate cells and several selenium-responsive genes have been identified that may be involved in cancer prevention. We will present data to show validation of array data, using quantitative real time RT-PCR, for one of the most significantly changing genes, selenoprotein W1 (SEPW1). SEPW1 mRNA levels significantly increased (> 3-fold) in response to a physiologically relevant dose of SeMC, which may confer increased cellular protection..

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INCREASING SELENIUM STATUS OF DAIRY COWS IN THE UK

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The use of dietary mineral supplements has increased in the UK dairy industry. From January 1995 to December 2004 blood samples from herds with possible trace-element responsive infertility have been sent to the University of Leeds for assessment of copper and selenium status. Selenium status was measured by automated enzymatic analysis (Cobas Mira) of erythrocyte glutathione peroxidase (GSH-Px) activity. The normalised data showed that there was a significant ($p < 0.001$) year on year increase in GSH-Px activities from 77 ± 31.9 U/ml PCV (mean ± SD) to 145 ± 50.7 U/ml PCV (n > 1500/year) in pre-treatment animals. Seasonal and geographic variations were less marked than the annual increase. Although the range of values encountered were similar, frequency distribution analysis showed an increasing proportion of animals with > 150 U/ml PCV, many times the acknowledged 'deficiency' level of 30 U/ml PCV, or 'adequate' level of 60 U/ml PCV. The highest levels are still below those indicating toxicity. There was no corresponding change in the mean copper status parameters (plasma copper, serum caeruloplasmin, erythrocyte super-oxide dismutase) and high selenium status did not correlate with copper status. The benefits from this change in the pre-treatment level of GSH-Px encountered in the UK dairy herd have yet to be evaluated.

THE PRESENCE OF THIOARSENOSUGARS IN SEAFOOD AND OTHER SAMPLES

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Inorganic arsenic is a well-known human carcinogen and, as a safeguard for human health, the WHO has recommended a maximum permissible level of 10 μg L-1 in drinking water. Arsenic also occurs in many foods, particularly in seafoods where it can be present at up to 100 μg g-1 (dry mass) or more. In many cases the majority of this seafood arsenic is present as arsenobetaine [$(CH_3)_3As^+CH_2COO^-$], a harmless compound which is rapidly excreted unchanged by humans. In the last year, however, a new group of arsenic compounds, namely thioarsenosugars, has been identified by HPLC/mass spectrometry in some marine samples, including seafood products. These compounds have unusual chromatographic properties which may have contributed to their remaining undetected until now. We have developed new chromatographic systems to facilitate the determination of thioarsenosugars, and our recent work has indicated that these compounds may be widespread in seafoods. The toxicology of thioarsenosugars remains unknown, and future assessment of the safety of food products in regard to arsenic and its compounds will need to address this issue. We present our latest results on the determination of thioarsenosugars in seafoods and related samples.

Funding

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CD, PB AND TRACE ELEMENTS LEVELS IN MATERNAL BLOOD, FETAL CORD BLOOD, AND PLACENTAL TISSUES IN JAPANESE PREGNANT WOMEN WHO SMOKE OR NOT SMOKE

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A cohort study was designed to examine the influences of perinatal exposures to heavy metals and other chemicals in Japanese children. In this study, we focused our examination on the maternal and fetal cadmium (Cd) and lead (Pb) levels, in relation to whether the mother smoked or not. One hundred and eight pregnant (non-smoker group: n = 54, sum of smoker and ex-smoker groups: n = 54) women participated in this study having given their written informed consent. Rice consumption and ages of participants were matched with both groups. Maternal peripheral blood, cord blood and placenta samples were collected for Cd, Pb and several trace elements analysis. Cd concentrations were determined with GFAAS. Pb and other trace elements (Cu, Zn, Mn) were determined by ICP-MS. Mean Cd concentrations of non-smokers were 1.29 ng/ml in maternal blood and 0.48 ng/ml in cord blood, those of smokers B (B-index 200<) were 1.52 ng/ml in maternal blood and 0.44 ng/ml in cord blood. Relationship between Cd, Pb, several trace elements levels and fetal development were discussed.

SEASONAL BLOOD LEAD AND 25-HYDROXY-VITAMIN-D CONCENTRATIONS IN CHILDREN

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We studied the effect of season on serum 25-hydroxy-vitamin-D (25-OH-D) and blood lead in seventy eight 1-3 year-old and sixty three 4-8 year-old urban children. Blood lead concentrations were 0.238 ± 0.190 and 0.315 ± 0.351 μmol/L during the winter and summer months for ages 1-3, a winter/summer (W/S) increase of 32.4%. There was a smaller W/S increase of 13.6% in blood lead from 0.177 ± 0.121 to 0.20 ± 0.140 μmol/L for ages 4-8. Ages 1-3 had serum 25-OH-D concentrations during the winter and summer of 99.3 ± 36.6 nmol/L and 100.8 ± 32.7 nmol/L, a 1.5% W/S increase. However, in ages 4-8 the W/S increase in 25-OH-D was from 74.1 ± 31.4 to 98.1 ± 28.2 nmol/L, an increase of 32.4%. The percentages of children with low (< 40 nmol/L) serum

25-OH-D concentrations were 8.5% in winter and 0.7% in summer. The large seasonal increase in blood lead in ages 1-3 was not accompanied by a significant increase in serum 25-OH-D concentrations. In contrast the seasonal increase in blood lead in ages 4-8 was accompanied by a large increase in serum 25-OH-D levels, and the increases were significantly associated ($r = 0.373$, $p = 0.0026$). The higher summertime 25-OH-D concentrations are likely due to increased sunlight-induced vitamin D synthesis and may contribute modestly to the seasonal increase in blood lead.

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EFFECT OF SELENIUM INTAKE AND FETAL AGE ON MRNA EXPRESSION OF TWO SELENOPROTEINS IN PORCINE FETAL AND MATERNAL LIVER.

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Activity of glutathione peroxidase-1 (GPX1) is markedly lower in liver of newborn pigs compared to fetal pigs at day 90 of gestation (Hostetler et al. 2004). The aim was to determine if mRNA expression changes during fetal development and if maternal Se intake affects mRNA expression. Prepubertal gilts (n = 42) were randomly assigned to either Se-adequate (0.39 ppm Se) or Se-deficient diets 6 weeks prior to breeding. Maternal and fetal liver was collected at d 10, 45, 70, and 114 (term) of pregnancy. Expression of mRNA for GPX1 and thioredoxin reductase (TRR) in fetal liver decreased 70% and 60%, respectively, between d 45 and 114 of pregnancy. Maternal Se intake did not affect fetal GPX1 or TRR mRNA expression. Low maternal Se intake decreased maternal GPX1 and TRR expression on d 10 and 45 of pregnancy but not d 114. In fact, compared to GPX1 and TRR expression on d 10 of pregnancy, dams fed the low Se diet had higher expression of GPX1 and TRR mRNA at term than those fed 0.39 ppm Se. In general, GPX1 mRNA expression agreed with GPX1 activity in liver of dams and fetuses except for increased expression at term in dams fed the low Se diet. These results support the hypothesis that neonatal pigs are born with a reduced oxidative defense compared to adults and that the maternal system mobilizes Se stores to maintain biochemical function of Se-dependent proteins.

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ZINC, COPPER AND MAGNESIUM AND RISKS FOR ALL-CAUSE, CANCER AND CARDIOVASCULAR MORTALITY-THE PARIS PROSPECTIVE STUDY II

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Experimental data suggest that zinc, copper and magnesium are involved in carcinogenesis and atherogenesis. Few longitudinal studies have related these minerals to cancer or cardiovascular disease mortality in population. Data from the Paris Prospective Study 2, a cohort of 4027 men aged 30-60 years at baseline, were used to assess the association between serum zinc, copper and magnesium and all-cause, cancer and cardiovascular disease mortality. During a 18-year follow-up, 327 deaths occurred; 175 due to cancer and 56 of cardiovascular origin. For each mineral, a reference group was defined in the statistical analyses: intermediate and higher values for serum zinc and magnesium (quartiles 2 to 4) and intermediate and lower values for serum copper (quartiles 1 to 3). All-cause and cancer mortality were associated to lower serum zinc (first quartile), lower serum magnesium (first quartile) and higher serum copper (fourth quartile) values, in age-adjusted Cox's model. After controlling for age, body mass index, smoking status, alcohol consumption, systolic blood pressure, serum LDL and HDL cholesterol, diabetes and cardiovascular disease history, lower serum magnesium and higher serum copper values were significantly related to increased all-cause and cancer mortality relative risks (RRs) by approximately 40 percent, compared to referent groups. Cardiovascular disease mortality was statistically significant increased

only for men with lower serum magnesium levels (multivariate RR = 1.7, 95 percent confidence interval (CI):1.0,3.0). Additionally subjects having both lower serum zinc and lower serum magnesium values had synergistically increased mortality risks from all-cause (RR = 2.1, 95 percent CI:1.5,2.9), cancer (RR = 2.3, 95 percent CI:1.5,3.6) and cardiovascular disease (RR = 2.7, 95 percent CI: 1.3,5.6). In conclusion, this large scale study shows that higher serum copper, lower serum magnesium and concomitance of lower serum zinc with lower serum magnesium levels contribute to an increased mortality risk in middle-aged men.

THE DIETARY ARSENIC INTAKES IN CHINA

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Purpose: In order to assess the safety of dietary arsenic intakes in different areas in China, we carried out Chinese total diet study in 2000 and obtained total arsenic and inorganic arsenic contents and intakes data. Method: Using the Chinese total dietary study method, the analytical samples were obtained in different areas by food consumption survey, food aggregation, food sampling and preparation. The levels of dietary total and inorganic arsenic were determined by the hydride generation atomic absorption spectrometry and atomic fluorospectrophotometry. The dietary total and inorganic arsenic intakes in different areas were obtained by timing the food consumption data and the arsenic content in different dietary samples. The safety of dietary arsenic was evaluated in four regions of Chinese and average adult intake calculated by using the dietary inorganic arsenic PTWI (define) recommended by WHO.

Result: The results indicate that the dietary arsenic intake is safe in the different regions. Only a few samples in some areas exceed the tolerance limits of China's national standard. Dietary total and inorganic arsenic (of PTWI) intakes in four different regions (north1, north2, south1, south2 and average adults) were 0.220 mg and 0.094 mg (69.3%), 0.254 mg and 0.098 mg (72.2%), 0.296 mg and 0.048 mg (35.6%), 0.335 mg and 0.077 mg (57.3%), 0.276 mg and 0.079 mg (58.6%) respectively. The main sources of dietary arsenic intake were cereals, vegetables, and beverages and water.

Conclusion: It is the first time that the dietary total arsenic and inorganic arsenic intakes of adults were investigated in a Chinese total diet study. The result shows that dietary arsenic intakes in Chinese people are safe.

ASSESSMENT OF SOME BLOOD PARAMETERS AS POTENTIAL MARKERS OF HEPATIC COPPER-ACCUMULATION IN CATTLE

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The aim of this study was to evaluate the suitability of various blood parameters as potential early markers of hepatic Cu accumulation in cattle. Paired liver and blood samples of 70 calves aged 6-10 months were obtained at slaughter in a region in NW Spain where animals usually have hepatic Cu concentrations above safe-adequate levels. Neither serum Cu concentration nor ceruloplasmin (CP) level, the two parameters most commonly used for diagnosis of Cu deficiency, were significantly associated with hepatic Cu concentration. However, whole-blood Cu concentration showed a slight but statistically significant correlation with hepatic Cu concentration ($r = 0.269$, $p = 0.026$). The use of calculated blood parameters, such as the serum or whole-blood non-CP Cu fraction, or CP/serum-Cu ratio, increased the correlation with hepatic Cu level ($r = 0.393$, $p = 0.001$), but the strength of the association remained insufficient for accurate prediction of hepatic Cu levels. Likewise, hepatic enzyme activities (AST and GGT) were significantly or nearly-significantly correlated with hepatic Cu levels, but again the strength of the association was too low for accurate prediction. We conclude that direct analysis of Cu levels in liver biopsies is the best technique currently available for detecting chronic subclinical Cu accumulation in cattle.

FACTORS AFFECTING COPPER ACCUMULATION IN CATTLE IN NW SPAIN

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Copper (Cu) metabolism in cattle is very complex and Cu accumulation in the body depends not only on total Cu concentration in the diet but also on other factors related to the diet and the individual animal. The aim of this study was to evaluate the influence of breed and other factors (age, sex and season) on Cu accumulation in calves in a region in NW Spain where there is much intensive pig farming and animals usually have hepatic Cu concentrations above the adequate levels. Paired liver and blood samples of 532 male and female Galician Blonde, Holstein Friesian and Galician Blonde x Holstein Friesian calves, aged 6-10 months, were obtained at slaughter. Samples were acid digested and Cu concentrations determined by ICP-OES. Holstein Friesian calves have significantly higher mean Cu concentrations both in the liver (80.6 mg/kg fresh weight) and blood (0.891 mg/l) than Galician Blonde (50.4 mg/kg and 0.748 mg/l) and crosses between both breeds (61.3 mg/kg and 0.829 mg/l). Hepatic Cu accumulation was significantly higher in males (63.8 mg/kg) than in females (53.5 mg/kg) and tended ($p = 0.110$) to increase with age. Calves slaughtered in winter showed significantly lower mean blood Cu concentrations (0.703 mg/l) compared to animals sampled in summer (0.940 mg/l).

EVALUATION OF BLOOD ELEMENTAL LEVELS AND APO E ALLELIC PROFILE IN HIGH RISK AND ATHEROSCLEROTIC SUBJECTS

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Atherosclerosis is a multifactorial disease influenced by factors such as genetic and metabolic ones. This study aims to evaluate and compare blood elemental levels (K, Ca, Fe, Cu, Zn, Se and Rb) and apo E allelic profile in subjects, aged 40-80 years, at different healthy conditions. Three groups were defined based on metabolic and clinical evaluation: a pathological group comprising patients suffering from stable atherosclerosis and under medical treatment; a group of subjects at high risk of atherosclerosis (hyper-lipidemic and -tensive); and a normo-tensive and –lipidemic group. Although no major discrepancies were observed for trace elements among the three groups, K levels were increased in plasma and blood cells while Ca levels were increased in plasma of patients, what might reflect an imbalance in electrolytic metabolism. The ε3 allele was the most frequent and the ε4 allele was found to be associated with the disease. This study contributes to a better understanding of the relationship between the apo E polymorphism and metabolic aspects in atherosclerosis, including those involved with the elemental metabolism.

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IRON, ZINC AND CALCIUM LEVELS IN COMPLEMENTARY FOODS CONSUMED BY INFANTS IN A RURAL REGION FROM NORTHERN ARGENTINA: PRESENT SITUATION AND PROPOSALS

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In Argentina, there is little information regarding the age of introduction of complementary foods as well as about their nutritional quality. The aim of this study was to assess the time of introduction and type and composition of foods fed to breastfed infants in a rural low-income community from Northern Argentina and to formulate alternative, nutritionally adequate gruels. Lactating women at 5, 6 and 7 months postpartum attending the paediatric control visits (Forres Hospital, Santiago del Estero) consented to participate and answered a structured questionnaire (n = 240 surveys). The usually consumed and the alternative gruels were prepared using local ingredients and analysed for macronutrients by AOAC (define) methods, and for iron, zinc and calcium by AAS (define). The mean age of introduction of gruels was 4.4 months. Most gruels had low energy density, low protein quality and inadequate levels of iron, zinc and calcium. The alternative gruels were designed according to WHO recommendations (World Health Organization,1998), using local, low-cost ingredients. They meet the mineral requirements for infants aged 5-7 months (DRI 2002), are compatible with local eating habits and economically feasible.

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IRON, ZINC AND CALCIUM LEVELS IN COMPLEMENTARY FOODS CONSUMED BY INFANTS IN A RURAL REGION FROM NORTHERN ARGENTINA: PRESENT SITUATION AND PROPOSALS

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ESTIMATION OF ZINC (ZN) REQUIREMENTS FOR SCHOOL-AGED CHILDREN IN THE WESTERN HIGHLANDS OF GUATEMALA

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Zn deficiency has been well documented in young children in the Western Highlands of Guatemala for whom maize is the major food staple and whose diets have high dietary phytate:Zn ratios. The objective of this study was to measure key variables of Zn homeostasis in pre-adolescent children in this population and to determine the effect of a modest reduction in dietary phytate:Zn molar ratio achieved with the substitution of a low-phytate maize for ten weeks. Data presented here are from the participants assigned to the local maize group. Fifteen of 20 subjects (8 male, 7 female, aged 6-12 years) in this group completed the study which required extrinsic labeling of all meals for one day with a Zn stable isotope and the intravenous administration of a second tracer followed by a period of urine and complete fecal collections, all in a rural village environment. Mean (± SD) results included: diet Zn: 9.1 (2.1) mg/d; phytate: Zn molar ratio: 23 (5); fractional absorption of Zn: 0.28 (0.04); absorbed Zn: 2.5 (0.5) mg/d; endogenous fecal Zn: 1.7 (0.6) mg/d; urine Zn: 0.2 (0.2) mg/d. Zn absorption was equal in 20 children randomized to the low-phytate maize group with dietary phytate:Zn ratio of 18:1 (5:1). Using a factorial approach based on these data, the estimated physiologic requirement was 2.1 mg Zn/d and the estimated average dietary requirement was 8.2 mg Zn/d which is higher than the DRIs of the Food and Nutrition Board for this age range.

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IDENTIFICATION OF NOVEL POLYMORPHISMS IN GENES INVOLVED IN SELENIUM-METABOLISM

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Selenium is essential for human health: severe deficiency is associated with cardiac dysfunction and sub-optimal intake increases risk of cancers; supplementation has been reported to reduce the progression of viral infection and cancer incidence. Individuals vary in their response to Se supplementation. Potentially, polymorphisms in selenoprotein genes involved in selenium transport and bioavailability could influence selenium metabolism and affect the response of individuals to selenium supplementation. In this study we screened cohorts of Caucasians, Chinese and South Asians for single nucleotide polymorphisms (SNP) in the gene regions corresponding to the coding region and 3'untranslated region of selenoprotein P and selenophosphate synthetase 2. Gene regions with potential polymorphisms were identified by Mutation Detection DNA-HPLC and then regions of heterozygosity were further analysed by sequencing in 50 individuals. Several novel polymorphisms were identified in both selenoprotein P and selenophosphate synthetase 2 genes. In some cases their frequencies differed with ethnicity. For example, the heterozygous and one homozygous variants of a SNP in the coding region of SelP gene occurred at approximately 46% frequency in Caucasians and Chinese but the homozygous variant predominated in South Asians. Both homozygous and heterozygous variants of a C->T SNP in the 3'UTR of selenophosphate synthetase 2 were frequent in South Asians but the CC genotype was predominant in Caucasians. The functional significance of these two SNPs is being determined in a supplementation trial in which individuals are supplemented with 100µg/day sodium selenite for 6 weeks and parameters of Se status measured.

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ZINC EFFECTS ON NUTRIENT-NUTRIENT INTERACTIONS AND TRENDS IN HEALTH AND AGEING: THE ZENITH STUDY

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Zinc is an essential trace element for human health and wellbeing. However, a moderate deficiency is often observed in elderly, even in developed countries. In order to evaluate the health effect of zinc supplementation in late middle-aged (55-70 years old) and older population (> 70 years old), a European project ZENITH was launched in 2002. This project will take place in parallel in 4 different European centres (Rome (Italy); Coleraine (Northern Ireland); Grenoble and Clermont-Ferrand (France)). This project is studying the effects of two nutritional levels of zinc supplementation (15 or 30 mg/day) during 6 months in older men and women, using a placebo controlled double-blinded design. Health zinc effects will be assessed on psychological and behavioural factors (changes in taste acuity, food choice, mood, cognitive function) and on surrogate biological markers (particularly antioxidants/ oxidative stress balance, immunity and thyroid functions, bone metabolism and protein synthesis). The absence of potentially toxic effects will also be evaluated on lipid profile and on the metabolism of other minerals and vitamins (iron, copper, folate, vitamins A and E). The data obtained should provide the basis for specific dietary recommendations for zinc intake in late middle-aged and older people. A large number of results are currently available and some of them will be presented during this conference in individual communications.

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CHROMIUM, NICKEL AND COPPER ACCUMULATION IN CATTLE RAISED IN A SERPENTINE-SOIL AREA IN GALICIA (NW SPAIN)

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Serpentine soils contain high amounts of chromium, nickel and copper. The aim of this study was to evaluate chromium, nickel and copper accumulation in cattle raised in a serpentine area in Galicia (NW Spain) where total and extractable chromium, nickel and copper concentrations exceed the thresholds recommended by safety standards and could be potentially toxic to plants and animals. Samples of liver, kidney and muscle of 41 animals from a serpentine-soil area and 15 animals from a control area were collected at slaughterhouses. Samples were acid-digested and metal concentrations determined by ICP-MS. Chromium and copper concentrations (geometric means) were significantly higher in cattle from the serpentine-soil area (Cr: liver 0.204, kidney: 0.209; Cu: liver: 75.9, kidney: 4.40, muscle: 1.73 mg/kg wet weight) than cattle from the control area (Cr: liver 0.139, kidney: 0.078; Cu: liver: 25.2, kidney: 3.58, muscle: 1.34 mg/kg wet weight). Although mean nickel concentrations were similar in both groups (liver: 0.022 and 0.023 mg/ kg, kidney: 0.161 and 0.236 mg/kg for cattle from the serpentine-soil and control area respectively), 20% of animals from the serpentine-soil area had high nickel accumulation in the kidney (12.9-17.6 mg/kg).

ORAL FERROUS SULPHATE TOLERANCE STUDY IN HEALTHY INDIVIDUALS

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Following oral supplementation with simple ferrous salts, two compartments have been especially identified that are associated with iron-related oxidative damage. The first is the gastrointestinal lumen (Lund et al. 1999) and may lead to acute gastrointestinal side effects, the second is the circulation where recent data show transient post-dose generation of non-transferrin bound iron (NTBI) (Hutchinson et al. 2004). Here, we have further assessed oxidative effects on the circulation and developed a gastrointestinal symptom diary for oral iron. In a double blind pilot study, 20 healthy volunteers were randomised to 400 mg per day oral ferrous sulphate (n = 10) or placebo (n = 10) for one week and they completed symptom diaries daily. Fasting blood samples were taken at baseline and four hours after the final tablet (i.e. day 0 and day 7), and assessed for NTBI and ascorbate levels. The mean number of symptoms were 5.5 times higher in the ferrous sulphate group than the placebo group ($p = 0.01$), mostly explained by heartburn and abdominal pain. Plasma NTBI was not significantly present at either time point and plasma ascorbate levels did not significantly rise (60.0 ± 23.7 and 71.7 ± 22.8 mmol/L pre and post ferrous sulphate respectively, $P = 0.2$). We conclude (i) the symptom diary appears responsive to iron-related gastrointestinal side effects for use in further studies and (ii) NTBI generation following oral ferrous salts either requires high iron absorption, as we showed previously with anaemic volunteers (Hutchinson et al. 2004) or is reduced following a period of iron supplementation. Further work will study these two possibilities.

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INTRACELLULAR LABILE ZINC AND ZINC TRANSPORTER LOCALIZATION IN MAMMALIAN CELLS AND IN YEAST

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Cellular zinc not tightly associated with metalloproteins is easily exchanged within the cell and concentrates to discrete subcellular pools (zincosomes). Cation Diffusion Facilitator (CDF) proteins, among which mammalian ZnTs and yeast Zrc1 and Cot1, are responsible for Zn efflux and intracellular compartmentalization. Using the Zn-specific fluorescent probe Zinquin and indirect immunofluorescence we sought to relate vescicular zinc transporters and zincosome formation. In the absence of added Zn, we observed the characteristic vesicular localization of labile zinc in two different mammalian cell lines. Increasing Zn up to 200 μM for 2 h, caused a decrease in zincosomal Zn and a parallel increase in a diffuse, cytoplasmic Zinquin fluorescence. The ZnT4 transporter did not display co-localization with zinc bodies before or after Zn addition. Further experiments are ongoing to compare the intracellular localization of other ZnTs in relation to zincosomes. In Saccharomyces cerevisiae labile zinc was visualized also by Zn-Se autometallography. Both techniques resulted in specific labelling of an intracellular vesicular compartment that was present in wild type cells as well as in the vacuolar Zn transporter mutants DELTA zrc1 and DELTA cot1. The vesicular compartment, that closely resembles mammalian zincosomes, appeared rapidly under conditions of zinc availability and was independent on endocytosis. However, persistence of the Zn loaded vesicles in nutritional zinc deficiency was dependent

on the presence of functional Zrc1 and Cot1 vacuolar transporters. Overall our findings indicate that labile zinc in yeast cells enters a dynamic vesicular compartment which could represent an extremely important defence to buffer nutritional Zn imbalance.

DIETARY SUPPLEMENTATION OF FARM ANIMALS´ DIET WITH ORGANIC SELENIUM: AN EFFECTIVE WAY TO NORMALIZE SELENIUM INTAKE IN MAN IN DEFICIENT COUNTRIES

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It is well known that many countries in the world are highly selenium deficient. To avoid selenium deficiency diseases in farm animals their diet is nowadays most often routinely supplemented with inorganic selenium as sodium selenite. Inorganic selenium compounds have – in comparison to organic compounds – a highly restricted capacity to increase the selenium content in foods of animal origin. As a consequence, the supplementation with selenite has only marginally increased the daily selenium intake in man; it is still insufficient in many parts of the world. The aim of the study was to quantify the consequence of a possible change from inorganic to organic selenium compounds - at comparable dosage levels of selenium - for supplementation of farm animals´ diet. Based on results from 16 scientific reports, the author has calculated that such a change can be expected to increase the selenium level in milk with least 100%, in cheese with at least 150%, in beef and pork meat with about 75%, in chicken meat with about 100%, in eggs with at least 100% and in liver with about 25%. With the eating habits of an average Swede, the total daily Se-intake will increase by at least 50%, thus changing the supply from being deficient to optimal. In countries with different eating habits, the magnitude of the influence on the total daily Se-intake might be different, but will certainly always be significant.

ATOMIC ABSORPTION SPECTROSCOPY FOR ZINC DETERMINATION IN HEPATIC TISSUE

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Alterations of hepatic zinc concentrations often occur in chronic liver disease.

Atomic Absorption Spectroscopy (AAS) is the preferencial method for metal determination.

The method validation is necessary in order to quantify the hepatic zinc concentration in liver tissue by AAS. The aim of this study is to validate this technique for the determination of zinc in liver tissue. Fragments of 30 bovine liver tissues were lyophilized, digested in nitric acid, placed in ultrasound for 60 min, and heated in stove at 60°C for 60 min. The analysis was carried out by AAS with graphite furnace (Perkin-Elmer model Analyst-300) using a zinc standard solution (National Institute of Standards and Technology). After construction of the calibration curves, 29 samples were analyzed according to validation protocols for bioanalysis assays of Sanitary Vigilance National Agency, International Conference on Harmonization of Food and Drug Administration. The results agreed with international norms: Variability coefficient for accuracy (% recovery of added zinc) < 15% and for precision (concordance and repeatability) +/- 10%. Linearity (relation between results and substance concentration) > 0.99 (r2- determination coefficient), concentration interval (interval between < and > values) from 2 to 64 ppb and quantification limit of 2 ppb. The method utilized for quantification of zinc in bovine hepatic tissue using AAS was valid. The determination of zinc in hepatic tissue may be utilized in the investigation of hepatic liver disease.

HEAVY METALS CONTENT IN CHONDROSTOMA WILLKOMMII FROM GUADIAMAR RIVER: IMPACT OF THE AZNALCOLLAR TOXIC SPILL

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The content of heavy metals was investigated in Chondrostoma willkommii, from the bed of Guadiamar river, after the Aznalcollar toxic spill. Sampling was done in 6 different dates, and in 2 different stations, affected and not affected by the spill. A total of 83 samples constituted of gill, liver, muscle, kidney, and carcass was studied. Mineral determinations were done using graphite furnace and flame atomic absorption spectroscopy. Results indicate that Cu content (mg/kg) ranged between 0.3-72.0 and 0.6-32.1 in not affected and affected stations, respectively. Fe content (mg/kg) in different studied organs varied between 3.2-225.6 in not affected and 14.0-3906.6 in affected station. 3.6-78.6 and 0.0-244.3 were intervals of Zn concentration found in samples from no affected and affected stations, respectively. Concentrations of Mn (mg/kg) varied between 0.0-8.7 in not affected and 0.0-26.1 in affected station. In the not affected station, content of Pb (µg/kg) ranged between 0.0-3123.4, while content of this mineral varied 118.0-63603.9 in the affected station. Cd (µg/kg) content ranged between 0.0-487.7 in not affected and 0.0-4243.2 in affected station. Pb content in gill was higher in the affected station than in the not affected station where a slight decrease was observed during the study period. In muscle, Pb content decreased in both stations but remarkably in the affected station. Cd content in gill was homogenous between stations and during the study period, except at 5th date of sampling. By the start of the study, Cd content in muscle was slightly lower in the not affected station.

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DETERMINATION OF HEAVY METALS CONTENT IN CARCASS OF SQUALIUS PYRENAICUS AND LEPOMIS GIBBOSSUS FROM GUADIAMAR RIVER AFTER THE AZNALCOLLAR TOXIC SPILL

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Content of Cu, Zn, Fe Mn, Pb and Cd was investigated in carcasses of 2 species of fish Squalius pyrenaicus and Lepomis gibbossus from the bed of Guadiamar river, after the Aznalcollar spill. Sampling was done on 6 different dates and in 5 different stations. Mineral determinations were determined in a total of 89 samples, taken from both species, using graphite furnace and flame atomic absorption spectroscopy. Main findings indicate that carcass content of Cu (mg/kg) ranged between 0.5-4.1 in Lepomis gibbossus and 0.7-8.4 in Squalius pyrenaicus. While content of Zn (mg/kg) varied between 11.8-75.6 and 29.0-97.1 in Lepomis gibbossus and Squalius pyrenaicus, respectively. In the carcasses of Lepomis gibbossus content of Pb (µg/kg) ranged between in 0.0-8792.2. Content of this mineral in the carcasses of Squalius pyrenaicus ranged between 0.0-2497 µg/kg. Cd content (µg/kg) ranged between 0.0-169.0 in Lepomis gibbossus and 4.6-26.6 in Squalius pyrenaicus. In the case of Lepomis gibbossus, Pb and Cd concentrations were higher in 2 of the affected stations in comparison with the not affected station. No remarkable evolution of Pb content was observed. In contrast, Cd concentrations tend to increase during the study period. In the case of Squalius pyrenaicus, Cd content was maintained at a same level in the affected station, while a slight increase in the non affected station during the study period was observed.

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INFLUENCE OF THE AZNALCOLLAR TOXIC SPILL ON HEAVY METALS CONTENT IN MICROPTERUS SALMOIDES FROM GUADIAMAR RIVER

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Content of Cu, Zn, Fe Mn, Pb and Cd was investigated in Micropterus salmoides from the bed of Guadiamar river, after the Aznalcollar toxic spill. Sampling was done in 5 different dates in a station located upstream from the toxic spill. A total of 51 samples constituted of gill, liver, muscle, kidney, and carcass was studied. Mineral determinations were made using graphite furnace and flame atomic absorption spectroscopy. Results show that Cu content (mg/kg) ranged between 0.6-3.3 in gill, 1.6-10.4 in liver, 0.3-4.2 in muscle, 3.5-20.0 in kidney and 0.5-0.8 in carcass. Content of Fe (mg/kg) varied between 28.6-71.6 in gill, 32.6-155.74 in liver, 1.5-5.1 in muscle 117.0-221.9 in kidney and 9.8-16.2 in carcass. Zn content (mg/kg) was varying between 12.8-21.96 in gill, 6.9-27.7 in liver, 3.6-7.8 in muscle 7.0-60.0 in kidney and 19.3-37.0 in carcass. In gill, liver, muscle, kidney and carcass, Mn content (mg/kg) varied between 0.5-4.4, 0.0-12.2, 0.0-0.3, 0.0-0.9 and 1.8-4.0, respectively. Content of Pb (μg/kg) ranged between 30.5-348.1 in gill, 0.0-102.7 in liver, 0.0-35.0 in muscle 0.0-74.3 in kidney and 0.0-0.0 in carcass. While Cd content (μg/kg) varied between 1.6-7922 in gill, 3.0-199.3 in liver, 0.0-2.3 in muscle 11.8-171.2 in kidney and 3.6-33.7 in carcass. A remarkable increase of Cd and Pb concentrations in gill, kidney, liver and muscle was observed at the 4th date of sampling.

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TOXIC AND ESSENTIAL TRACE ELEMENTS IN BLOOD AND URINE FROM SLOVAK POPULATIONS FROM AREAS EXPOSED TO INCREASED LEVELS OF PCBS

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PCB Risk: Evaluating human health risk from low-dose and long-term PCB exposure, is a project examining the long term health effects of exposure to PCBs and other organohalogen pollutants in a polluted region of Eastern Slovakia. More than 1000 age and sex matched adults and 250 children have been examined. Exposure to trace elements has been measured in a sub group of 300 adults and all the children in blood and urine samples by DRC ICPMS (define). Hg, Pb Cd, Sb, Bi and U were measured in standard mode, Fe, Cr, Mn, Ni, Cu, Zn Se and Co in DRC mode using NH_3 as cell gas and As in DRC mode using O_2 as cell gas. For most elements the observed concentrations agree with published reference ranges for other European populations. No differences were observed between subjects living in the polluted area and in a control area. There was no correlation of element concentrations with blood concentrations of organic pollutants. Increased urine uranium concentrations were seen in some subjects from the exposed population. Uranium exposure many be due to employment at the manufacturing site or to exposure from other sources.

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THE ASSOCIATION BETWEEN THE G277S MUTATION OF THE TRANSFERRIN GENE AND IRON DEFICIENCY ANAEMIA

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Several studies have addressed the genetic disorders of iron overload but less have focused on iron deficiency anaemia. Transferrin is the most important carrier of iron in blood plasma. A case of atransferrinemia has been described (Beutler et al. 2000), characterized by microcytic anaemia and by hepatic iron overload, which presented mutations in the exons 5, 12 and 13 of the transferrin gene. Lee et al. (2001) found that a polymorphism in exon 7 (G277S) of the transferrin gene, was associated with a reduction in total iron binding capacity which predisposes menstruating women to iron deficiency anaemia. However, in vitro studies (Aisen, 2003) show that the G277S mutation does not disturb transferrin function. Our team recruited a group of 162 premenopausal women, aged 18-45 years, non-pregnant, non-smokers, not diagnosed with thalassaemia nor haemocromatosis. Serum ferritin, haemoglobin concentration, haematocrit, and mean corpuscular volume were determined. DNA was extracted and analysed for the G277S, C282Y and H63D mutations. Sixteen women presented G277G/G277S (3 heterozygotes and 1 homozygote for H63D). The remaining 146 were G277G/G277G (8 C282Y heterozygotes and for H63D, 49 heterozygotes and 6 homozygotes). Among G277G/G277S and G277G/G277G volunteers, 18.8% and 13% presented iron deficiency anaemia (Hb <120g/L and ferritin< 20 μg/l), respectively. Results do not show an association between the presence of the G277S mutation of the transferrin gene and iron deficiency anaemia in the study group of women.

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A METHOD FOR MEASURING LIPID-SOLUBLE ARSENIC SPECIES PRESENT IN CRUDE FISH OILS, FISH OIL SUPPLEMENTS AND FOODSTUFFS

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Lipid-soluble arsenic compounds (arsenolipids) occur in a wide range of biological samples including many common foodstuffs. The study of these compounds has been hindered, however, by the lack of a suitable analytical technique able to separate and measure the various lipid species. As a source of arsenolipids, we used 10 crude fish oils from various regions of the world. Total arsenic analyses on the fish oils, performed with Inductively Coupled Plasma Mass Spectrometry (ICPMS), gave concentrations from 4.3 to 10.5 mg As kg-1. Analysis of the fish oils for arsenolipids was performed by normal phase HPLC-ICPMS with various mixtures of organic solvents as mobile phases. Inherent problems of instability associated with the introduction of organic solvents to the plasma were overcome by the use of reduced column flow, a chilled spray chamber, and the addition of oxygen directly to the plasma. All ten fish oils appeared to contain the same 4-6 major arsenolipids, but in varying amounts depending on the origin of the fish. Quantification was achieved by external calibration against triphenylarsine oxide or triphenylarsine sulfide, and the sum of species following HPLC of the oils matched well the total arsenic results (92-107%). The method was applied to samples of food supplements (fish oil capsules) and a packaged food product (cod liver) whereby arsenolipids were measured and found to be significant arsenic constituents. This study represents the first attempt to directly measure arsenolipids present in biological samples.

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INVESTIGATION OF IMMUNOTOXICITY AND ALLERGENIC PROPERTIES OF POTASSIUM, MAGNESIUM, ZINC AND CHROMIUM ASPARTATES

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Trace element imbalances are known to be the causes of many pathologies in human organism. Now for the treatment of trace elements metabolism deviations various mineral complexes are used. The present work shows the results of pre-clinical investigation of K, Mg, Zn and Cr aspartates, aimed at estimation of their potential negative influence on human organism. In experiments on CBA and C57BL/6 mice, CBA-C57BL/6 F1 hybrids and albino guinea pigs (all males) immunotoxic and allergenic properties of the above compounds were studied. After immunization of the animals by K, Mg, Zn and Cr aspartates combined with mycobacterial adjuvant no delayed-type allergy reactions were observed in sites of the s.c. injections. Also, the compounds caused no significant changes of phagocytic index or cell immunity parameters. Fortnight peroral administration of the K, Mg, Zn, Cr aspartates did not significantly influence the weight of thymus, spleen and popliteal lymph nodes as estimated by routine methods, indicating absence of toxic effect of the substances on lymphoid tissue cells. It was found that the aspartates suppressed inflammatory reaction induced by concanavalin A, the Mg aspartate causing the maximal effect. In addition, Mg aspartate suppressed systemic anaphylactic reaction of the organism to ovalbumin; though the other aspartates caused no significant influence on intensity of the systemic anaphylaxis. Fortnight administration of the aspartates was also found to decrease chemiluminescent response of mice's neutrophils to opsonized zymosan. Thus, the obtained data evidenced that aspartates of K, Mg, Zn and Cr had no immunotoxic or allergenic influence on the organism, but possessed anti-inflammatory and, possibly, antioxidative properties.

EVALUATION OF NUTRITIONAL OXIDATIVE STATUS AND ZINC IN TYPE 2 DIABETICS

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Type 2 diabetes is a major cause of vascular complications. Generally, zinc status is decreased in most type 2 diabetes patients. Zinc is required for structural and functional integrity of several transcription factors and more than 300 enzymes. Zinc plays an important role in processes associated with oxidative stress. There is some evidence that this trace element can support indirect antioxidant functions and can protect and stabilize biological membrane. This study was designed to evaluate the nutritional status of zinc and its relations with some parameters of oxidation in patients with diabetes type 2. Blood samples were also collected to determine concentrations of zinc, superoxide dismutase activity (SOD), lipid profile and oxysterols. There were assessment anthropometric measurements by Body Mass Index (BMI). The mean age was 58 years. The 8.5% of the participants were classified as normal according to the BMI results. The median of zinc concentration in plasma and erythrocyte was, 70.8 µg/dL and 41.1 µg/gHb, respectively, despite 66% and 44.7%, being below the reference values. There is a positive correlation with zinc in plasma and triglycerides and zinc in erythrocyte with triol ($p < 0.01$). The activity of SOD was normal in 61.7% of the participants, although most of this doesn´t have normal parameters in zinc concentration.

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SEASONAL VARIATION IN HEPATIC COPPER CONCENTRATIONS IN A SHEEP FLOCK WITH CHRONIC COPPER POISONING PROBLEMS

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In Norway, chronic copper poisoning is usually a spontaneous disease in adult ewes, primarily in the autumn. The background is hepatic copper accumulation, because of low molybdenum levels in mountain pastures (Garmo et al. 1986). In a flock of about 50 ewes, with severe losses from chronic copper poisoning, a two-year study was done on the seasonal variation of hepatic copper concentrations in individual ewes. Liver biopsies were taken out in the field, immediately frozen in liquid nitrogen, and kept frozen until analysis with AAS. Each ewe was sampled in December, March, June and October. A complete set of biopsies was obtained from 14 ewes each year, and from 5 ewes through both years. Hepatic copper concentrations varied from 16 to 400 µg/g ww. Substantial differences were found between the copper levels in different ewes, and these differences remained throughout the study. In each ewe, concentrations changed only moderately from October till March, fell significantly from March to June, and rose again from June to October. The results may explain the seasonal occurrence of chronic copper poisoning, and show large individual differences in susceptibility.

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COMPARATIVE ANALYSIS OF CHANGES IN HAIR AND URINE ELEMENTAL CONTENT AT EXCESS PB, CD, AS, NI INTAKE IN HUMANS

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In the present work adequacy of hair analysis as an assessment tool for risk of intoxication with chemical elements was estimated and relation between elemental content of hair and urine was studied. Simultaneous investigation of Pb, Cd, As and Ni content of hair and urine of 20 glass factory workers and 13 tannery workers was carried out using ICP-MS method. Also comparison of the data of hair and urine elemental analysis was made between two groups: the control (Pb, Cd, As or Ni content of hair was within normal range; n = 53) and the experimental one (Pb, Cd, As or Ni content of hair was above biologically allowable level; n = 6 to 16 for different elements). Differences in frequencies of toxic elements accumulation in hair and urine were estimated using chi-square criterion. It was found that excess Pb and As intake connected with occupational contact of the examined persons with metals leads to simultaneous increase of their levels in hair (48% and 24%, Pb and As respectively) and urine (30%). At the same time, increased Cd and Ni levels were found in hair of examined people but no cases of increase of these elements in urine were observed. Estimation of contingency of changes in Pb, Cd, As and Ni content of hair and whole blood by Fisher exact test revealed that increase of Pb and As level in hair was reliably accompanied by growth of their level in urine ($p < 0.05$). No relation between increasing levels of As, Ni in hair and urine was found because no one case of increased As, Ni content of urine was detected. Thus, significant relation between excess accumulation of toxic element in hair and urine was established for lead and arsenic, and not established for cadmium and nickel.

COMPARATIVE ANALYSIS OF CHANGES IN HAIR AND WHOLE BLOOD ELEMENTAL CONTENT AT EXCESS PB, CD, AS, NI INTAKE IN HUMANS

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The work was devoted to studying adequacy of hair analysis as an assessment tool for risk of intoxication with chemical elements and relation between elemental content of hair and whole blood. Simultaneous investigation of Pb, Cd, As and Ni content of hair and whole blood of 20 glass factory workers, 14 tannery workers and 10 children residing in a sanitary area of a lead-zinc processing plant was carried out using ICP-MS method. Also comparison

of the data of hair and whole blood elemental analysis was made between two groups: the control (Pb, Cd, As or Ni content of hair was within normal range; n = 45) and the experimental one (Pb, Cd, As or Ni content of hair was above biologically allowable level; n = 10 to 25 for different elements). Differences in frequencies of toxic elements accumulation in hair and whole blood were estimated using chi-square criterion. It was found that contact with Pb was equally reflected in its content of both hair and whole blood (57% of examined persons) while Cd exposure was reflected by whole blood better than by hair (66% and 23%, respectively). Increase of As, Ni content of whole blood at their excess intake into the organism was poorly expressed and significantly more rarely detected as compared to that in hair (18% vs. 2% and 18% vs. 5% for As and Ni in hair and blood accordingly). Analysis of Pb, Cd, As and Ni content of hair and whole blood by Fisher exact test revealed significant contingency of changes in the element content of hair with the changes in its content of whole blood for Pb and Cd ($p < 0.001$). For As and Ni the analogous dependence was not found. Thus, significant relation between excess accumulation of toxic elements in hair and whole blood was established for lead and cadmium, and not established for arsenic and nickel.

THE IODINE STATUS OF NEW ZEALAND CHILDREN

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The first Children's Nutrition Survey (NZCNS) in New Zealand (NZ) was conducted in 2002. The NZCNS was a cross-sectional survey of a nationally representative sample of school children aged 5-14 years. Blood drawn from an antecubital vein and a casual morning urine sample was obtained from 1154 children. Thyroid Stimulating Hormone (TSH) and Thyroglobulin (Tg) concentration was determined in serum samples, and free tri-iodothyronine (ft3) and free thyroxine (ft4) concentration was determined in plasma samples, using immunoassay techniques. Urinary iodine concentration (UIC) was determined using a modification of the Sandell-Kolthoff reaction. The median UIC of the children was 67 μg/L, and 28% of the children had a UIC < 50 μg/L, indicative of mild iodine deficiency according to the International Council for the Control of Iodine Deficiency Disorders (ICCIDD). The concentrations (mean ± SEM) of TSH (1.72 ± 1.70 mU/L), fT3 (6.0 ± 0.0 pmol/L), and fT4 (14.9 ± 0.2 pmol/L) were similar to values published for children in other countries. The median Tg concentration was 12.8 ng/mL and fell within the range of 10-20 ng/mL, indicative of mild iodine deficiency according to ICCIDD. Furthermore, children who had an UIC < 50 μg/L had a significantly higher ($p = 0.000$) serum Tg concentration than children with a UIC above this level, suggesting hyperplasia of the thyroid gland in children with lower UIC. These data clearly illustrate that NZ school children have mild iodine deficiency, and that the level of iodine in their diet needs to be increased.

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ORGANIC SELENIUM SUPPLEMENTATION OF DAIRY COWS REDUCES HYDROGEN PEROXIDE-MEDIATED OXIDATION OF PROTEINS IN MILK

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Selenium (Se) supplementation of dairy cows in the form of the natural amino acid selenomethionine, provided as the yeast product SelPlex™ at 25 mg or 100 mg Se per cow per day, allowed steady-state levels of 100 or 300 mikrogram Se per litre of milk to be achieved within two days. This Se-enriched milk had lower formation of dityrosine, a specific protein oxidation product, following oxidative challenge with hydrogen peroxide. Cu^{2+}, another pro-oxidant, was unable to form dityrosine. The lower formation of dityrosine in the Se-enriched milk probably occurred as a consequence of non-specific incorporation of selenomethionine in place of methionine in the milk. Purification of lactoperoxidase, a methionine-rich enzyme catalysing hydrogen peroxide-mediated dityrosine formation, from the Se-supplemented milk indicated a novel protein band compared with lactoperoxidase purified from control milk. We hypothesize that incorporation of selenomethionine residues into milk

proteins provides novel antioxidant properties that may be exploited to produce dairy consumables of superior quality and stability.

Funding

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THE EFFECT OF SE-METHYLSELENOCYSTEINE SUPPLEMENTATION ON GLUTATHIONE PEROXIDASE MRNA AND PROTEIN LEVELS IN PROSTATE CELLS

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Selenium is essential for the maintenance of human health through its integration into selenoproteins, deficiencies of which may contribute to health problems including impairment of immune function, susceptibility to viral diseases and cancer. Glutathione peroxidase (GPx) is a selenoenzyme that catalyses the reduction of hydroperoxides in the presence of glutathione, thus protecting various cellular components from oxidative damage. One important bioactive form of selenium found in Se-enriched vegetables including garlic, broccoli and onions is Se-methylselenocysteine (SeMC). The aim of this study was to determine the effect of SeMC on selenoprotein expression. Prostate cells (LNCaP clone FGC and PNT1A) were cultured in the presence of various doses of SeMC ranging from deficiency (20nM) to supra-nutritional levels (3000nM) to establish any alterations in GPx1 mRNA and protein levels that may occur over this range. The cells were adapted to the relevant SeMC concentration for one month prior to harvesting at which point Western blot analysis was carried out to measure protein levels and RT-PCR techniques were used to quantify mRNA levels. Differences were observed in response to SeMC supplementation between the two cell lines. Data will be presented to show glutathione peroxidase mRNA and relative protein levels.

Funding

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SELENIUM AND IODINE DEPLETION DIFFERENTIALLY AFFECT GROWTH AND DENSITY, MICROARCHITECTURE, AND STRENGTH OF BONE IN GROWING MALE AND FEMALE RATS

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Epidemiological studies suggest a relationship between a combined deficiency of selenium (Se) and iodine (I) and impaired bone health. This project investigated the effects of experimental Se and/or I depletion on bone strength and structure in growing rats. Dams were fed experimental diets in a 2 × 2 factorial arrangement (+Se+I; +Se-I; -Se+I; -Se-I), beginning at week 1 of lactation. Pups were weaned at 3 weeks of age and a sub-sample of males and females were fed the experimental diet of their mothers for an additional 7 weeks. Se depletion in the pups was confirmed by liver glutathione peroxidase activity and I deficiency by serum thyroxin and thyroid weight. Males grew more rapidly than females but weight gain was reduced by Se depletion in males. I depletion reduced weight in all animals. Se depletion decreased both serum osteocalcin and ferric reducing ability of plasma (FRAP). Both Se and I depletion decreased bone mineral area, content, and density as measured by dual energy x-ray absorptiometry (DEXA). Three-D analysis of proximal tibia and the third lumbar vertebra (L_3) by micro-computed tomography (μCT) showed lower bone volume/total volume (BV/TV) in males than females. In Se-depleted animals BV/TV was higher when iodine was adequate. Trabecular number, thickness and connectivity were higher in females than males and in Se-depleted animals the values were higher when iodine was adequate. Bone area and trabecular separation (TbSp) were higher in males than females. When Se was deficient, TbSp was reduced by I. Finite element analysis of L_3 trabecular cores by (μCT) showed forces to compress; average strain and stiffness were higher in females than males. Selenium and I depletion impair growth and density, microarchitecture and strength of bone in growing rats.

Funding

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DEVELOPMENTS IN THE MINERAL NUTRITION OF POULTRY

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Intensified poultry meat and egg production has lead to health, welfare and pollution problems, several being related to mineral nutrition. Current recommendations are based on inorganic mineral sulphate and oxide supplements used in trials run in the 1950-60s, irrelevant to modern feeds, genotypes and performance. Recently, chelated peptide mineral supplements, akin to organic forms found in plant and animal tissue, have been developed that are more digestible, efficiently absorbed and utilised. Canadian research demonstrated that only 20% of the recommended mineral levels were required from organic sources to maintain health and growth in broiler chickens (Leeson, 2005). In Europe, raw material mineral levels are typically lower than North America, requiring higher feed supplementation, and increasing pollution risk. Spanish replicated studies, using 12 birds in 8 replicates, substituted organic for inorganic minerals in feed and found significantly ($p < 0.05$) improved growth performance at 66% organic mineral replacement. Initial data indicated reduced mineral excretion. Commercial 48-60 week old layers fed organic manganese and zinc had 4% increased structural (Eshell) eggshell integrity, 8% less shell variability, with 22% fewer cracked table eggs. Similar US trials showed 5% higher eggshell strength. Improving supply of dietary minerals can maintain or increase poultry growth at lower supplementation levels, benefit eggshell quality and reduce pollution via manure disposal.

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BENEFITS OF ORGANIC MINERAL SUPPLEMENTATION IN DOMESTIC DOGS

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Since 1990, commercial chelated mineral products have been developed that are more readily digested, preferentially absorbed and better utilised in animals compared to standard inorganic mineral sulphates and oxides. Reported benefits of organic minerals include reduced supplementation requirements and excretion, improved reproductive and immune status. Research with dogs has shown that organic supplementation doubled zinc deposition ($P < 0.05$) in 10 cm2 hair over 25 d (Lowe et al, 1994). Lowe & Wiseman (1998) found that feeding organic zinc reduced losses through excretion by 10%, and increased retention of both copper and zinc in tissues of dogs. Trials recently conducted at the University of Vienna measured the faecal digestibility of copper, manganese and zinc fed in either organic or inorganic forms. Forty adult beagles, housed in a randomised block over two time periods, received either a control diet with no added minerals, 100% inorganic minerals at common commercial levels, 66% inorganic/33% organic, 33% inorganic/66% organic or 100% organic minerals. 14 d faecal collection was made following 14 d adaptation period. Results are expected to confirm significant reductions in faecal mineral excretion.

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TRACE ELEMENT CONTENT AND REDOX BIOMARKERS IN COPD

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Chronic Obstructive Pulmonary Disease (COPD) is a highly prevalent disease characterized by airflow limitation and an abnormal inflammatory response. Cigarette smoking is the major environmental risk factor. Two main mechanisms are assumed in COPD pathogenesis: oxidative stress resulting from an oxidant/antioxidant equilibrium impairment, and a proteinase-antiproteinase imbalance. The objective of this work was to study blood biochemical parameters related to oxidant damage and antioxidant status in COPD patients. Plasma and blood cells element concentrations (K, Ca, Fe, Cu, Zn, Se, Rb), plasma protein carbonyls, whole blood glutathione peroxidase (GPx) and erythrocytes superoxide dismutase (SOD) activities were chosen as blood biomarkers of electrolytic and redox balance. Plasma selenium concentrations were lower in patients, especially in those with low arterial oxygen pressure (<70 mm Hg), while K and Rb were significantly increased in their blood cells. Plasma protein carbonyls were also increased in patients whereas GPx and SOD activities were higher and lower than controls, respectively. For COPD patients, no significant changes were found in smokers relative to ex-smokers. These changes may reflect altered electrolytic homeostasis and oxidant damage and can be interpreted as markers of COPD, rather than indicators of smoking habits.

Funding

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STUDIES ON THE NEGATIVE EFFECTS ON LONG TERM SODIUM SELENITE TREATMENT OF DOWN SYNDROME AND JUVENILE NEURONAL CEROID PATIENTS (JNCL)

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Because of pharmacologically beneficial effects of inorganic selenium in animals, we have given sodium selenite to humans suffering from Down´s syndrome (DS), and juvenile neuronal ceroid lipofuscinosis. In DS elevated formation of H2O2 due to over expression of SOD-1 results in free radical stress. The supplementation 0.015-0.025 mg Se/kg/d in the form of sodium selenite increased the E-GSH-Px activity by 28%, and decreased the SOD/GSHPx ratio by 23.9% ($p < 0.01$); decreased the elevated Cu level in blood mononuclear cells. Steady state level of E-GSHPx was reached when s-Se level approached 150 ug/L. The CuZn SOD-1 level in JNCL patients has been reported to be elevated. About 200 Finns have been diagnosed with JNCL, a fatal progressive disease of the CNS (define). Today 31 out of a total of 60 alive JNCL patients are supplemented with 0.05 mg Se/kg/d as sodium selenite. The toxic range has been considered 4 uM/l for serum Se according to our studies. Although the duration of Se supplementation has been more than 10 years, the mean serum level increases approximately from about 70 ug/l to 300 ug/l. In a few patients a slight increase in serum aspartate aminotransferase activity has been observed. The dental caries or gingivitis was not observed, neither was chromosomal aberration or sister-chromatid exchanges observed in patients during one year of supplementation.

DETERMINATION OF ELEMENT BINDING PATTERN IN CYTOSOLS OF CULTURED LUNG AND TRACHEA CELLS AFTER TREATMENT WITH SE OR AS USING SIZE EXCLUSION CHROMATOGRAPHY HYPHENATED TO PLASMA MASS SPECTROMETRY

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The respiratory system, especially the trachea and the lungs, are strongly exposed to environmental hazards. Pollutants as well as the necessary oxygen induce oxidative stress in the cells of the epithelial layer. The metabolism of occurring oxidative species is often related to metalloproteins, particularly to metal depending enzymes. Two different respiratory cell types (human trachea and lung cancer cells) as well as a liver cell line were cultured with and without additional selenium or arsenic. The aim of this pilot study was to examine where these elements are bound to and what are the differences in the binding pattern of other elements. Cell culture conditions, cytosol extraction and instrumental setup were optimised for the detection of the element binding pattern. SEC-ICP-MS (define) investigations of cytosols from arsenic treated cells show two major As-containing compounds in the lung cells (one in the low molecular region and one at about 8 kDa), whereas the trachea cells have an additional high molecular As-binding compound at ~75kDa. Differences in the element profiles of copper and zinc are also visible between the As (+) and As (-) treated cells and will be shown in this presentation.

THE SEARCH FOR COPPER BIOMARKERS IN HUMANS: USE OF IN VITRO CELL SYSTEMS TO DETECT CANDIDATE REGULATOR GENES OF COPPER HOMEOSTASIS

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Despite copper being an important cofactor in many different biological processes, suitable biomarkers that predict the copper status of an individual have yet to be identified, which makes dietary recommendations difficult to set. To compound this, there is little information on the genetic control of the homeostatic mechanisms regulating copper status. The aim of the project is to define the genetic regulation of copper homeostasis using in vitro cell systems representative of key tissues (liver and intestine) involved in copper homeostasis, and to identify copper status biomarkers. By utilising lymphocytes, as these are readily accessible in human studies, it is hoped that the markers present in peripheral blood can be used as surrogates for the copper-induced effects in remote target tissues. The in vitro cell culture system has been optimised using a low oxygen environment in order to more accurately mimic the physiological conditions of the cells in vivo condition. By combining this design with transcriptomics and proteomic technologies, time and dose dependent affects of copper on the gene expression profiles of candidate regulated genes in these systems, are currently being assessed. The outcome of this project should provide a mechanistic understanding of copper homeostasis and the means of determining and/or predicting deficiency or overload in individuals. Preliminary data will be presented outlining dose-dependent effects of copper on cell growth, viability and gene expression.

EVALUATION OF 41CA AS A NOVEL ISOTOPIC TOOL IN BONE RESEARCH

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Progress in osteoporosis prevention is limited by the availability of suitable techniques to measure changes in bone metabolism directly and on a short-term scale. Isotopic labeling of bone Ca may overcome current limitations. Once bone Ca is labeled, changes in urinary tracer excretion should directly reflect changes in bone Ca metabolism. Isotopic labeling of bone became possible using 41 Ca, a very long living radioisotope, which is rather inexpensive and can be administered at negligible health risk by using ultra-sensitive mass spectrometric techniques (AMS, RIMS). 24 post-menopausal women received an oral dose of 41 Ca (100 nCi) for isotopic labeling of bone Ca. Labeled subjects participated in a bisphosphonate intervention (risedronate, 6 months) to evaluate induced changes in 41 Ca excretion against changes in bone mineral density (define) (n = 6). Remaining subjects (n = 16) participated in a randomized cross-over, placebo controlled Ca supplementation trial (750 mg Ca/d, 3 mo) to evaluate the sensitivity of the technique. Isotopic labeling was found to be complete ca. 200 days post dose. Both interventions were effective. Bisphosphonate treatment increased BMD significantly for spine (+3.0%, $p = 0.01$). Ca supplementation resulted, in a significant lowering in D-Pyr (bone resorption marker; -19.4%, $p = 0.04$) and BAP (bone formation marker; - 7.2%, $p = 0.04$). Changes in urinary 41Ca excretion paralleled findings made by conventional techniques. Bisphosphonate treatment resulted in a change in Ca transfer rate from the slow into the fast exchanging pool by -56% ($P < 0.0005$) indicating a lowering of bone resorption. Ca supplementation had no significant effect on Ca transfer rates between the slow and the fast exchanging pool but altered Ca transfer rate from the fast exchanging pool to plasma by -31% ($P < 0.0005$). The sensitivity of the new technique, however, was much higher when compared to established methods. Changes in 41 Ca excretion could be identified unambiguously in each individual in less than a month.

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COXSACKIEVIRUS B3 INFECTION IN KESHAN DISEASE PATIENTS AT A LOW SELENIUM AREA OF CHINA

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Keshan disease (KD) is an endemic cardiomyopathy that only occurs in selenium deficient areas of China. Coxsackievirus B3 (CVB3) infection has been proposed as a co-factor for the etiology of KD under the condition of selenium deficiency. The aim was to find out whether or not the infection of Coxsackievirus B3 is involved in the etiology of KD. The blood samples were taken from 30 KD patients and 30 healthy people from normal selenium areas as control. LDE-PCR (long distance enterovirus-specific RTPCR) for enteroviruses, two specific ELISAs for CVB1-6 IgM and CVB1-6 IgG, and three-primer RTPCR specific for CVB3 were used. Results show that: 1) The infection rate of enteroviruses in blood from KD patients is higher than that from control (80% vs. 0%, $P<0.01$); 2) The CVB1-6 antibody positive rate in blood from KD patients is higher than that from control (IgM:33.3% vs. 0%, IgG:23.3% vs. 0%, $P < 0.01$); 3) 16.6% of enteroviruses positive samples or 40% of CVB1-6 IgM antibody positive samples can be identified as CVB3. So we may consider that the persistent infection of enteroviruses might be involved in the pathogenesis of KD, but we are not sure whether the cardiovirulent CVB3 are from the mutation of CVB3/0 under the condition of selenium deficiency.

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INTERACTIONS OF IODINE DEFICIENCY AND VITAMIN A DEFICIENCY: EFFECTS ON THYROID FUNCTION IN CHILDREN

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In developing countries, children are at high risk for both the iodine deficiency disorders (IDD) and vitamin A deficiency (VAD). The study aim was to determine the effect of VAD and vitamin A (VA) supplementation on thyroid function in an area of severe iodine deficiency and endemic goiter. In a double-blind, randomized, 10-mo trial, Moroccan children with IDD and VAD (n = 138) were given iodized salt and either VA (200,000 IU) or placebo at 0 and 5 months. At 0, 5 and 10 months, measurements of VA status and thyroid function were done. At baseline, increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH and thyroglobulin ($P < 0.001$). In children with VAD, the odds ratio (OR) [95% CI] for goiter was 6.51 [2.94, 14.41]. VAD severity was also a strong predictor of higher concentrations of TT4 ($P < 0.001$); the OR [95% CI] for hypothyroidism in VAD was 0.06 [0.03, 0.14]. During the intervention, mean thyroglobulin, median TSH and the goiter rate significantly decreased in VA-treated group compared to placebo ($P < 0.01$). The findings indicate that VAD in severely-IDD-affected children increases TSH stimulation and thyroid size, and reduces risk for hypothyroidism. This effect could be due to decreased VA-mediated suppression of the pituitary TSHβ gene. In IDD and VAD-affected children receiving iodized salt, concurrent VA supplementation improves iodine efficacy.

Funding

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SERUM TRANSFERRIN RECEPTOR AND ZINC PROTOPORPHYRIN AS INDICATORS OF IRON STATUS IN AFRICAN CHILDREN

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Although transferrin receptor (TfR) and zinc protoporphyrin (ZnPP) are often used to define iron status in school-age children in developing countries, diagnostic cut-offs for this age group are uncertain. The objective was to determine the sensitivity and specificity of TfR and ZnPP in predicting iron deficiency (ID) in black and white children in Africa. Hemoglobin (Hb), C-reactive protein (CRP), serum ferritin (SF), TfR, and ZnPP were measured in children in Ivory Coast and Morocco. We excluded children with an elevated CRP, and then used receiver operating characteristic (ROC) curves to evaluate TfR and ZnPP alone and in combination in screening for ID, defined as a SF < 15 μg/L, and iron deficiency anemia (IDA), defined as a SF < 15 μg/L and a low Hb. The sample included 2814 children aged 5-15years. Sensitivity and specificity of TfR and ZnPP were limited by considerable overlap between iron-sufficient, nonanemic children and those with IDA. Based on the ROC curves, we identified diagnostic cut-offs for TfR and ZnPP that achieved specificities and sensitivities of ≈ 60-80%. Separate cut-offs for Ivory Coast and Morocco gave the best performance; the cut-offs for both TfR and ZnPP were higher in Ivory Coast. Moreover, comparing nonanemic, iron sufficient subjects, Ivorian children had significantly higher TfR and ZnPP than Moroccan children ($p < 0.01$). In conclusion, new diagnostic cut-offs for TfR and ZnPP based on ROC curve analyses may improve their performance in defining iron status in children. There are significant ethnic differences in TfR and ZnPP suggesting that separate cut-offs may be needed for black and white children.

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IRON DEFICIENCY DUE TO CONSUMPTION OF A HABITUAL DIET LOW IN BIOAVAILABLE IRON: A LONGITUDINAL COHORT STUDY IN MOROCCAN CHILDREN

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In many developing countries, cereal and legume-based diets contain low levels of bioavailable iron, which may increase risk of iron deficiency. The study aim was to measure iron nutrition in Moroccan children consuming their habitual diet containing low amounts of bioavailable iron. The study was a prospective, longitudinal free-living cohort study in iron-replete, non-anemic 6-10 year-old children (n = 126). Hemoglobin, serum ferritin and transferrin receptor were measured at baseline. The children then consumed their habitual cereal and legume-based diet for 15 months, when their iron status was retested. Using weighed food records and direct food analysis, dietary iron intake and iron bioavailability were calculated. Based on the change in hemoglobin and body iron stores calculated from the serum ferritin/transferrin receptor ratio, iron balance and iron absorption were estimated over the 15 month period. Mean daily iron intake was 10.8 mg/d, 97% of which was non-heme iron. Estimated nonheme iron bioavailability from algorithms was 1.0-4.3% adjusted for low body iron stores. Over 15 months, the mean change in total body iron was -142 mg, and mean iron absorption was estimated to be 0.22 mg per day, or 2% of dietary iron. Mean hemoglobin concentration fell 12 g/L. At 15 months, 75% of the cohort had deficits in tissue iron, and 1/3rd had mild iron-deficiency anemia. In conclusion, low iron bioavailability from legume and cereal-based diets is a cause of iron deficiency in children in rural Africa.

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COPPER LEVELS MEASUREMENTS IN THE CEREBRO ESPINAL FLUID

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The hidrocephalia is a condition with high morbimortality. The election treatment is the Peritoneal Derivated Valve (PDV) and the principal complication of this procedure is the infection of the valve. The concentrations of copper in the cerebro espinal fluid (CSF) from patients with infected Peritoneal Derivated Valve (IPDV)and from patients with PDV no infected (control group) were measured by Atomic Absorption Spectrometry before antibiotic treatment(day 1) and post antibiotic treatment(day 21).The aim was to examine the effects of the infection on CSF levels. Subjects (n = 15) with IPDV had CSF copper levels significantly elevated (26.3 ± 4.3 mcg/l) compared to the levels of the control group(n = 10) who had (20.13 ± 3.7 mcg/l).There were no significant differences between IPDV copper levels (19.1 ± 2.8 mcg/l) after 21 days antibiotics treatment and the control group copper levels. The physiological basis for the differences in copper CSF levels between IPDV patients and control group is unknown but could be related to alterations of the blood brain barrier.

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ZINC AND AGE-RELATED DECLINE IN TASTE ACUITY

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Taste acuity declines with age potentially bringing about changes in diet that have implications for health, wellbeing and quality of life in older people. Zinc has been shown to improve ability to perceive sweet, bitter

and sour taste in deficient individuals. It is therefore possible that supplemented zinc or zinc rich foods could compensate for and correct deficiencies associated with decline in the sense of taste. The EU-funded Zenith project appears to be the only placebo controlled clinical trial that has explored taste acuity in response to zinc in healthy older individuals. Healthy older people (n = 101) were recruited from within community organisations in Northern Ireland and supplemented with placebo, 15 mg or 30 mg zinc. Sensitivity to the four basic tastes (sweet, sour, salty and bitter) was assessed using a three-alternative, forced choice, signal detection approach. In contradiction to previous research employing clinical populations, zinc sulphate supplementation up to 30 mgs did not appear to enhance taste perception in healthy older people. These preliminary results suggest that age-related detriment in taste sensitivity is not the result of increased zinc requirement.

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ZINC CONTENT OF COW MILK IS AFFECTED BY DRY PERIOD CALCIUM AND PHOSPHORUS FEEDING

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Milk is an important nutrient to the newborn calf as well as man ('s children). The mineral and trace element content in milk is generally high – especially in colostrum – and of a high availability in the absorption processes.

The present study followed 20 cows 6 weeks prior to calving until 3 weeks after calving. All animals were fed according to Danish Feed Recommendations for dry cows (pregnant), however four groups were allocated based on this, i.e. 1) no further supplements, 2) extra P-supplements, 3) extra Ca-supplements, and 4) extra P and Ca supplements in a 2 by 2 trial. After parturition all animals were fed equally. Milk was collected from day 0 to day 25 in lactation. Inorganic constituents (ash), calcium, magnesium, phosphorous and zinc was determined in the milk samples (n = 197). Colostrum (day 0-3) was higher in mineral constituents than later fractions (day 4-25). Ash, Ca, P, Mg and Zn content were 46, 65, 89, 199 and 234% higher in colostrums, respectively. Mg, P, and Zn contents in colostrums were lowered by dry period extra calcium dosage, i.e. 334 vs. 270; 1883 vs.1674; and 17.6 vs. 13.8 mg/kg, respectively (n = 44, $p < 0.05$). Colostrums Ca was not affected significantly. Mineral content of milk day 4-25 in lactation was generally affected by both Ca and P feeding in the dry period (Ca*P, $p < 0.05 – 0.001$). The dry period feeding effect on milk is likely to be founded in bone accretion and resorption before calving.

PLASMA COPPER, HYDROXYPROLINE AND OSTEOCALCIN IN COWS AROUND PARTURITION

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The connection between copper status and bone turnover has within the last decades attracted some attention. The present study describes 20 cows in the period 3 weeks before parturition to 3 weeks after. Basic feed rations were similar and according to recommendations. However, four groups were allocated, 1) no extra supplementation 2) extra P in feed 3) extra Ca in feed 4) extra P and Ca in feed before calving (only). Plasma osteocalcin, hydroxyproline and elementary copper were analysed during the period. Supplementation of only calcium before calving had no effect on blood hydroxyproline or Cu, but a significantly depressing effect on circulating osteocalcin ($p < 0.01$). The Ca-effect on post calving osteocalcin was even more pronounced ($p < 0.001$). P-supplementation before calving had a slightly depressing effect on hydroxyproline and Cu but a tremendous positive effect on plasma osteocalcin (35% increase, $p < 0.001$) before calving - not after ($p = 0.60$). Plasma osteocalcin and Cu appeared significantly inversely correlated before calving ($r = -0.19$, $n = 170$, $p < 0.05$) as well as after calving ($r = -0.24$, $n = 150$, $p < 0.001$). Cows belonging to group 1 and 4 were low in OH-proline before calving but increased markedly after calving ($p < 0.001$). Cows belonging to group 2 and 4 were high in plasma osteocalcin before calving but decreased significantly after calving ($p < 0.001$). All groups increased in plasma Cu after parturition ($p < 0.001$).

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