11th International Symposium on Trace Elements in Man and Animals Abstracts

001.—Trace Elements and Nitric Oxide Function. Michael A. Marletta. Department of Chemistry and Department of Molecular & Cell Biology, University of California, Berkeley, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, Division of Physical Biosciences, Lawrence Berkeley National Laboratory.

Nitric oxide (NO) has emerged over the last 15 years as a mammalian metabolic intermediate involved in the regulation of critical physiological functions such as blood vessel homeostasis, neuronal transmission and host response to infection. NO is synthesized by the enzyme nitric oxide synthase which converts the amino acid l-arginine to citrulline and NO. NO functions in biological systems in two very important ways. First it has been found to be a messenger by which cells communicate with one another (signal transduction) and secondly has been found to play a critical role in the host response to infection. In the host response to infection, it appears that the toxic properties of NO have been harnessed by the immune system to kill or at least slow the growth of invading organisms. The non-specific chemical reactivity with key cellular targets is responsible for this action. In signaling, NO directly activates the enzyme guanylate cyclase (sGC). Once activated, sGC converts GTP to cGMP and pyrophosphate. The cGMP formed is responsible for the well-documented actions of NO such as blood vessel dilation. With the initial discovery of NO signaling, several important questions emerged that centered largely on the issue of how a signaling system functions when the signaling agent is chemically reactive (short-lived), highly diffusible, and toxic. Critical, especially in signaling, is the control of NO biosynthesis and interaction with the biological receptors at a concentration that will not harm the host. Why did nature choose NO for the roles it has? That question engenders only speculation. How does NO work (i.e. what does NO do and how does it do it without harm and with specificity)? Answers to those questions can now be offered as an interesting picture of molecular level details emerges.

002.—Scientific Research: Essential but is it Enough to Combat World Food Insecurities? Barbara A. Underwood. Food and Nutrition Board, Institute of Medicine, National Academies, Washington DC, USA.

Micronutrients are front stage in basic science laboratories where researchers are elucidating their roles on genetic expression and influences on metabolism and functions in plants, animals and humans. Epidemiological studies have established associations of micronutrient deficiencies and excesses with health and development outcomes, some of which have been confirmed by clinical intervention trials using single or multiple micronutrient supplements. The agricultural community is challenged through selective breeding and genetic modifications to improve density and bioavailability of micronutrient in staple crops, while not sacrificing yields. The private sector is prodded to provide micronutrient-fortified foods that reach the poor while minimizing their profit margins. Poor households are encouraged through education to diversify diets through home gardens and small animal husbandry to supplement staples in their family's diet. Yet, success in translating scientific research, epidemiological findings and education to acceptable efficacious intervention that address global micronutrient problems depends on a receptive social, economic and political environment. Too frequently that framework is developed internationally and/or nationally targeted to desired recipients. Experience shows that technically focused interventions that ignore appropriate empowerment processes are less likely to be successful. Those processes enable changes in people's perceptions, motivations and behaviors. Sustainability is enhanced when the viewpoint of local consumers (not to be considered only recipients) participate in identifying local resources, needs and social, economic and political barriers. Scientific research is crucial, but partnerships for attaining micronutrient adequacy at all levels are critical if sustained control is the goal.

003.—Human Zinc Homeostasis: Good But Not Perfect. Michael Hambidge. Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO.

Currently, there is an apparent dichotomy between the extensively documented occurrence of human zinc deficiency and the widely held perception that regulation of human zinc homeostasis is effective in maintaining zinc balance, including appropriate retention, over a wide range of dietary zinc intake. Factors that contribute to this dichotomy include: (1) the effect of dietary inhibitors, notably phytic acid, which have an adverse effect on the bioavailability of dietary zinc; (2) losses of endogenous zinc that are disproportionately high in relation to the quantity of zinc absorbed or/and to zinc status. Examples of both excessive losses and of conservation of endogenous zinc are considered in relation to a template that accounts for the interrelationship between these endogenous zinc losses and the quantity of zinc absorbed; (3) imperfect regulation of zinc homeostasis which maybe inadequate to protect from some extent of zinc depletion when dietary zinc is low. In addressing these factors, special consideration will be given to relevant research of the author and his colleagues, including data derived

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from studies in communities whose habitual diet is low in zinc and/or high in phytic acid.

005.—Trace Metals and Host Defense—Recent Advances and Continuing Challenges. Mark L. Failla. Department of Human Nutrition, The Ohio State University, Columbus, OH, U.S.A.

Experimental and clinical investigations during the last three decades of the 20th Century clearly showed that a) both severe deficiencies and excesses of essential trace elements impair the ability of the mammalian host to combat infectious pathogens, and b) well regulated changes in the transport and metabolism of trace metals represent an integral component of the host response to invasion. Indeed, trace element malnutrition increases the incidence, duration and severity of viral and microbial infections. Results from some, but not all, intervention trials in developing countries support the benefits of zinc and iron supplementation for the prevention and reduction in severity of selective infectious diseases. While generally assumed that these changes are due to suppressed activities of the immune cells, the elegant studies of Beck and associates have demonstrated that selenium deficiency can enhance virulence of the infectious agent by inducing alterations in the viral genome. This discovery raises the possibility that deficiencies and excesses of other trace elements can improve the fitness of the parasite in the hostile environment of the host, as well as compromise the functional integrity of the immune system. Many descriptive roles of the trace elements in various processes associated with the development, maturation, activation and effector activities of immune cells have been characterized. The application of modern analytical techniques to problems in nutritional immunology is beginning to yield new insights about the molecular functions of trace elements in the normal regulation of immune cell development and response to infectious stimuli. In order to translate discoveries about trace element-dependent modulation of signaling pathways and responses of immune cells to stimuli into effective preventative and therapeutic strategies, the availability of reliable and sensitive and indicators of essential trace metal status is required. Examination of the possibility that cellular and molecular functions of the trace elements in host defense may in turn provide sensitive biomarkers for the assessment of nutritional status also merits consideration. Selective examples of recent discoveries about the cellular and molecular functions of trace elements in host defense and their possible application to the problem of assessment will be discussed.

006.—Cellular Response Against Oxidative and Nitrosative Stress: Role of Selenium, Tellurium, Zinc and Copper. Lars-Oliver Klotz, Klaus-D. Kröncke,* Elena A. Ostrakhovitch, Darius P. Buchczyk and Helmut Sies. Institut für Physiologische Chemie I and *Research Group Immunobiology, Biomedical Research Center, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany.

Glutathione peroxidases (GPx) are selenoenzymes well known for catalyzing the reduction of hydroperoxides at the expense of glutathione. Cytosolic GPx has been shown to also catalyze the reduction of peroxynitrite, thus acting as a peroxynitrite reductase. We postulate that selenoenzymes such as GPx, selenoprotein P and thioredoxin reductase can serve as a first line of defense against oxidative and nitrosative stress. Both enzymatic activities of GPx are mimicked by low-molecular-weight compounds containing selenium (e.g. ebselen) or tellurium. Reduced metallothionein may also serve as source for the reducing equivalents required for the reactions catalysed by selenium or tellurium compounds, with Zn2+ being released from metallothionein during its peroxidation. Zinc ions may also be released from other intracellular sources during oxidative and nitrosative stress: peroxynitrite exposure of Zn2+-finger transcription factors such as vitamin D receptor/retinoid X receptor heterodimers impairs their biological activity, probably by oxidation of the Zn2+-finger cysteines. Thus, peroxynitrite may affect cellular signal transduction processes. Growth factor receptors are targets for peroxynitrite-modulated signaling as well, leading to activation of the antiapoptotic phosphoinositide-3 kinase/Akt pathway. Copper ions are another potent activator of this Akt pathway, demonstrating that cellular responses to different stressful stimuli may converge in the activation of final common signaling cascades regulating cellular survival. This establishes a second cellular line of defense against oxidative and nitrosative stress, with a pivotal role of metal ions.

007.—Metalloneurobiology: Lessons from the Zinc Front. Christopher Frederickson. NeuroBioTex Inc. and The University of Texas Medical Branch, Galveston TX 77550

"Metalloneurobiology" is a term coined by Dr. SJ Lippard (MIT), to identify a new frontier in biology. This frontier bridges aspects of traditional synaptic transmitter neurochemistry, second- and third-messenger intracellular signaling, metalloenzymology, and trace metal nutrition. In the case of zinc metalloneurobiology, much of the descriptive phenomenology has been completed, and a rough outline of the physiological and pathological aspects of zinc signaling are coming into focus. Thus, we know that certain neurons sequester mM concentrations of weakly-bound, rapidly-exchangeable ("free") Zn2+ ions in their presynaptic vesicles and release the ions synaptically, generating brief "puffs" of "free" zinc ion in the extracellular space. Post synaptic targets of the released zinc ions are also established, and the functional consequences of blocking the zinc signals are coming under scrutiny. Most intriguingly, it is now fairly certain that some of the zinc that is released synaptically actually crosses synaptic clefts and enters post-synaptic neurons, through identified, gated, zinc-permeable channels. In physiological amounts, this trans-cellular zinc signal appears to mediate a form of synaptic learning, and the enzymology of these effects is now at issue. In pathological amounts, the same zinc is cytolethal. Zinc metalloneurobiology has progressed in the main by carefully reviewing the history of calcium neurobiology. Many of the theoretical and methodological lessons learned will doubtless be useful to those who blaze trails in copper, rubidium, manganese, or molybdenum metalloneurobiology.

010.—Zinc-altered Immune Function. Lothar Rink and Klaus-Helge Ibs. Institute of Immunology, University Hospital, Technical University of Aachen, Aachen, Germany.

Zinc is an essential trace element for the immune system. The immune system as a highly proliferating cell system strictly depends on the availability of zinc. The role of zinc within the immune system has been studied during the last decades in vivo

and in vitro. However, the molecular basis of zinc action is still poorly understood. During the last years it was shown that high dosages of zinc induced immune defects similar to this of zinc deficiency. Therefore zinc has to be balanced to save a powerful immune function. However, the cells of the immune system are differently affected by zinc. Even minute alterations in the zinc level influences T cell development as well as T cell functions, whereas granulocytes are only influenced by high dosage of zinc or profound zinc deficiency. Interestingly, monocytes are the only leukocytes which are directly activated by zinc. As granulocytes they show a high tolerance to extracellular zinc. The influence of zinc on B cells is more complicated and depends on the differentiation grade of the cells. Cellular functions of the cells, especially the cytokine production is also modulated by zinc. However, the modulation of cellular functions is often influenced by the alterations of the immunostimulants by zinc. Therefore, in vitro results have to be proven before they could be transferred to the in vivo situation. Furthermore, the in vitro effective dosage of zinc is difficult to reach in in vivo experiments.

However, in some groups like elderly persons and hemodialysis patients, the immune defects are comparable to those induced by zinc deficiency. Therefore zinc may be a simple and cheap way to improve the immune status of a number of persons which have a high risks of infections.

012.—Selenium in the Immune System. John R. Arthur, Roderick C. MacKenzie* and Geoffrey J. Beckett.[+] Division of Cellular Integrity, Rowett Research Institute, Bucksburn, Aberdeen, Scotland, AB21 9SB, *Department of Medical and Radiological Sciences, University of Edinburgh, Edinburgh, Scotland, EH3 9YW and [+]Department of Clinical Biochemistry, University of Edinburgh, Edinburgh, Scotland, EH3 9YW.

Selenium has many biological functions mediated through an array of selenoproteins. These proteins can influence a range of biochemical systems in the body including those involved in antioxidant mechanisms, thyroid hormone metabolism and redox control. All these processes can impinge on elements of the immune system and it is therefore not surprising that selenium can influence both the humoral and cell mediated immune responses. In addition, dietary selenium intake can influence eicosanoid metabolism and modulate the balance between polyunsaturated fatty acid metabolites that may promote inflammatory processes with differing potencies. Since many cell-mediated immune processes involved reactive oxygen-derived species, these have the potential to damage host cells as well as the foreign organisms that are targeted by the immune process. Thus there is evidence that immune cells can damage themselves when there is insufficient selenium to protect against free radical species generated in response to infective challenges. The glutathione peroxidases are likely to play a role in the protective systems since, peroxides are generated by the cells during these responses. However, since the characterisation of other selenoproteins such as thioredoxin reductases, selenoprotein P and low molecular weight selenoproteins the mechanisms whereby selenium influences the immune systems should be reassessed. For example, there is now evidence that selenium can affect aspects of responses that do not rely upon generation of oxygen-derived free radicals, consistent with function of non-peroxidase selenoproteins.

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013.—Bcl-2 Regulates Zinc Induced Caspase 9 Mediated Apoptosis. James J. Mann, Jr.* and Pamela J. Fraker.[+] *Cell and Molecular Biology Program and [+]Department of Biochemistry and Molecular Biology. Michigan State University, East Lansing, Michigan, 48824.

Growing evidence suggests that free zinc can induce apoptosis in many types of cells including cells from the immune and nervous systems. Neurons damaged by brain ischemia release zinc, which appears to be associated with apoptosis seen in surrounding cells. Understanding this 'bystander' apoptosis in which zinc is released into the surrounding environment perhaps playing a role in promoting cell death could lead to improvements in the management of various diseases. An ionophore, zinc pyrithione was used at very low (nanomolar) concentrations in order to study the pathways zinc utilizes to induce apoptosis in mouse thymocytes. The heavy metal chelator N,N,N′,N′-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN) inhibited zinc pyrithione induced apoptosis while sodium pyrithione, at equivalent concentrations failed to induce apoptosis. These and other controls suggested that it was indeed zinc initiating apoptosis that was dependent on both transcription and translation. Many apoptotic cascades employ the Bcl-2 regulated (caspase 9 mediated) mitochondrial apoptotic pathway as the point where death stimuli proceed from initiation into execution. Zinc induced apoptosis was inhibited by the broad-spectrum caspase inhibitor Z-VAD-FMK as well as the specific caspase 9 and 3 inhibitors. Furthermore, overexpression of Bcl-2/Bcl-xl in Ramos or Jurkat cell lines inhibited zinc induced apoptosis. Other signaling pathways, including PKC__ appear to be involved and are currently under investigation. This work clearly demonstrates that zinc pyrithione at nanomolar concentrations utilizes a Bcl-2 regulated caspase 9 mediated pathway to induce apoptosis. Supported by NIH, DK52289.

014.—Effects of Zinc Deficiency and Cortisol on the Oxidative Burst Activity of Human U937 Cells Differentiated by 1,25-Dihydroxyvitamin D3 (VD) Plus Granulocyte-monocyte Colony Stimulating Factor (GM-CSF). Zhixin L. Huang and Pamela J. Fraker. Michigan State University, Department of Biochemistry and Molecular Biology, East Lansing, MI.

Our previous studies indicate that Zinc (Zn) deficiency and increased levels of glucocorticoids, conditions existing alone or in combination during malnutrition, impair the differentiation of human U937 promonocytic cells. The current research examined the effects of Zn deficiency and cortisol (Cs) on the oxidative burst activity, one of the defense responses of monocytes. U937 cells were grown in Chelex 100-treated Zn deficient (ZD) and/or Cs (0.1_M)-containing medium for 2 d followed by another 2 d of differentiation with 10 nM VD plus 0.5 ng/ml GM-CSF. The exposure to either ZD or Cs medium significantly decreased cell proliferation and differentiation in comparison with the controls. In the PMA-induced oxidative burst, Zn deficiency increased the secretion of superoxide anion (O◊-) of U937 monocytic cells by 44% (P £ 0.05) while Cs decreased this activity by 53% (P £ 0.05), as measured spectrophotometrically by the reduction of ferricytochrome c.

The combination of Zn deficiency and Cs treatment did not alter ($P \geq 0.05$) the secretion of O◊-. In addition, none of these treatments caused any appreciable decrease in intracellular reactive oxygen species, as determined flow cytometrically by the oxidation of 6-carboxy-2′7′-dichlorofluorescein diacetate. The increased O◊- secretion by ZD U937 monocytic cells suggests that the monocytes attempt to adapt to Zn deficiency and maintain their host defense capacity by upregulating survival mechanisms.

015.—Ferroportin-1 (Fpn1) and Iron Release by a Murine Macrophage Cell Line. Mitchell Knutson and Marianne Wessling-Resnick. Harvard School of Public Health, Nutrition Dept., Boston, MA.

Recent studies implicate Fpn11 (also known as IREG-12 and MTP13) in the export of iron from the duodenal enterocyte into the portal circulation. The abundance of Fpn1 transcripts in macrophages 1,3, coupled with the observation that iron induces macrophage Fpn1 mRNA synthesis4, suggests that it may also function to export iron from these cells. The release of iron from macrophages, which recycle iron from senescent erythrocytes, represents the largest iron efflux pathway in the body. To examine Fpn1 activity in macrophages, J774 cells were incubated with opsonized erythrocytes (EIgG) for 2 h. Phagocytosis was stopped by removing noningested EIgG, and subsequent changes in Fpn1 mRNA levels were measured by Northern analysis. Iron release was measured by gamma counting of 59Fe in the culture medium after phagocytosis of 59Fe-labeled EIgG. The results of these experiments demonstrate that after erythrophagocytosis, Fpn1 mRNA levels increase in a time-dependent manner and are 10-fold greater at 6–8 h, but decline to basal levels by 18 h. The increase in Fpn1 mRNA levels depends upon celluar iron content because the addition of salicylaldehyde isonicotinoyl hydrazone, a lipophilic iron chelator, suppresses this induction. After erythophagocytosis of 59Fe-labeled EIgG, the amount of 59Fe in the culture medium increases over time, and approximately 10% of the 59Fe obtained via erythrophagocytosis is released from J774 cells after 8h. However, the rate of 59Fe export is greatest 2 h after erythrophagocytosis and declines thereafter. Thus, the observation that increasing Fpn1 mRNA levels are associated with decreasing rates of iron release raises questions about the possible role of Fpn1 in the export of iron acquired via erythrophagocytosis.

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016.—The Effect of a Molybdenum or Iron Induced Copper Deficiency on Cellular and Humoral Immune Responses in Growing Lambs. Claire L. Williams, Robert G. Wilkinson and Alexander M. Mackenzie. ASRC, School of Agriculture, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK.

Copper deficiency in ruminants is induced by high dietary intakes of molybdenum (Mo), iron (Fe) or sulfur (S) and has been associated with increased disease susceptibility. The aim of this study was to assess the effects of Mo or Fe and S on the immune function of growing lambs. Twenty-four Charollais X Friesland lambs were randomly allocated to one of three dietary treatment groups with eight lambs per group. All lambs were fed ad libitum a basal complete diet for thirteen weeks. Group one received the basal diet (control). In addition to the basal diet, group two received an additional 500 mg/kg DM Fe and 2 g/kg DM S (Fe) and group three received an additional 5 mg/kg DM Mo and 2 g/kg DM S (Mo). Blood samples were taken and assessed for superoxide dismutase (SOD) and ceruloplasmin (CP) activity. During week 4 and 8 of the trial, all lambs were immunised with 1 mg of Keyhole Limpet Haemocyanin (KLH). Lamb anti-KLH IgG and IgM responses were measured by direct ELISA. Lymphocyte blastogenesis was assessed using the mitogens PWM, Con A and KLH. Results indicated that the CP activity of the control group was significantly higher compared with the Mo and Fe groups ($p < 0.05$) up to week 4. However, after week 4 there was no significant differences in the mean CP activities between the treatments, although by week 12, mean CP was lowest in the Mo group (control 22.8 mg/dL, Fe 17.4 mg/dL, 15.9 g/dL). SOD activity was significantly higher ($p < 0.01$) in the control group compared with the Fe and Mo group (respectively) during weeks 5, 7, 9 and 12. SOD activity showed that there was a continuous trend for the Mo group to be lower than the control or Fe treatment groups. There were no significant differences between dietary treatments on mitogen stimulated lymphocyte blastogenic response, or between treatments for anti-KLH IgG or IgM responses. It can be concluded that thiomolybdate molecules which are absorbed into the blood and tissues may subsequently inhibit copper enzyme activity. A reduction in antioxidant status (CP and SOD) may increase susceptibility to infection over time. There was no clear effect on a Fe or Mo induced copper deficiency on specific immune response in lambs, as has previously been reported.

018.—The Cellular Zinc and Redox States Converge in the Metallothionein/Thionein Pair. Wolfgang Maret. Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Cambridge, MA.

The paramount importance of zinc (Zn) for a wide range of biological functions is based on its occurrence in thousands of known Zn proteins. In order to regulate the availability of Zn dynamically, eukaryotes have compartmentalized Zn and the metallothionein/thionein (MT/T) pair, which controls the pico- to nanomolar concentrations of metabolically active cellular Zn. Interactions of Zn with sulfur ligands of donors turn out to be critical both for tight binding and creation of a reactive coordination environment from which the redox-inert Zn can be distributed. Biological oxidants such as disulfides and S-nitrosothiols oxidize the Zn/thiolate clusters in MT with concomitant Zn release. In addition, selenium (Se) compounds that have the capacity to form selenol(ate)s catalytically couple with the glutathione/glutathione disulfide and MT/T redox pairs to either release or bind Zn. In this pathway, Se expresses its antioxidant effects through redox catalysis in Zn metabolism. Se affects the amount and redox state of T, an endogenous chelating agent with twenty cysteines that contribute significantly to the thiol redox buffering capacity of the cell. Thus, hitherto unknown interactions between the essential micronutrients Zn and Se on the one hand and zinc and redox metabolism on the other are key features of the cellular

homeostatic zinc system. (Supported by the Endowment for Research in Human Biology, Inc.)

020.—Selenium Deficiency Increases the Risk of Viral Infections. Melinda A. Beck. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Keshan Disease (KD), an endemic cardiomyopathy first reported in China, was found to affect individuals who were deficient in selenium (Se). Because KD had a seasonal and annual incidence, an infectious co-factor, possibly a coxsackievirus, was suspected along with a deficiency in Se. Using a mouse model to mimic the conditions of KD, Se-deficient and Se-adequate mice were infected with an amyocarditic strain of coxsackievirus. Infected Se-deficient mice developed myocarditis, whereas Se-adequate mice did not develop any heart pathology. Passaging the virus recovered from the Se-deficient mice back into Se-adequate mice resulted in the development of myocarditis in the Se-adequate mice. The change in virulence of the amyocarditic virus was found to be due to changes in the viral genome. Six nucleotide changes were found in the virus that replicated in the Se-deficient mice. None of these changes occurred in virus that replicated in Se-adequate mice. Once the mutations occurred, even mice with normal Se status were vulnerable to the newly mutated virus. Virus replicating in mice fed a diet deficient in vitamin E or with excess iron will also develop mutations, suggesting that the driving force for the viral mutations is increased oxidative stress of the host. The ability of the nutritional status of the host to influence a viral genome was not restricted to coxsackievirus. Se-deficient mice infected with a mild strain of influenza virus developed much more severe lung pathology as compared with infected Se-adequate mice. As for coxsackievirus, the influenza virus genome mutated in the Se-deficient host, changing a normally mild influenza virus into a much more virulent one. Thus, the nutritional status of the host not only affects the host, but directly affects the genome of viral pathogens as well.

021.—Novel Mammalian Selenoproteins: Identification and Studies on Their Characteristics and Functions. Dietrich Behne and Antonios Kyriakopoulos. Hahn-Meitner-Institut Berlin, Dept. Trace Element Research in the Life Sciences, Glienicker Str. 100, D-14109 Berlin, Germany.

After labeling rats in vivo with Se-75 and gel electrophoretic protein, more than 35 Se-containing proteins or protein subunits with molecular masses between 3 and 116 kDa could be distinguished. Among those, proteins with molecular masses of 15 and 34 kDa were chosen for more detailed investigations. Previous studies on rats had revealed the existence of a 15 kDa selenoprotein with a native molecular mass of about 250 kDa and a pI value of about 4.5–4.7 which is enriched in the prostatic epithelium (1). A 15 kDa selenoprotein with similar characteristics was later identified in human T-cells (2). After separation of rat tissue homogenates by two dimensional IEF/SDS-PAGE, three selenocysteine-containing spots with pI values of 4.5, 4.7 and 7.0 were found in the 15 kDa range. MALDI-MS showed that the two acid protein spots stemmed from the known protein and that the rat 15 kDa selenoprotein and the human 15 kDa selenoprotein are the same compound in two species. The selenocysteine-containing spot with a pI value of 7.0, however, did not react with an antibody raised against this protein and was found to represent a novel 15 kDa selenoprotein with a native molecular mass in the range of 30 kDa. It is enriched in the brain and might have important functions in the central nervous system. In order to be able to distinguish the two selenoproteins we have named them according to the differences in their pI values as 'acid 15 kDa selenoprotein' and 'neutral 15 kDa selenoprotein'. A further selenoprotein with a molecular mass of 34 kDa, has now been identified as a specific sperm nuclei glutathione peroxidase (snGPx). It is expressed in the late spermatids by alternative splicing of the PHGPx gene and differs from PHGPx in its N-terminal sequence which contains a nuclear localization signal and an arginine-rich region by which it is most probably attached to the DNA. The enzyme is located in the sperm nuclei where it is the only selenoprotein present. Its appearance coincides with the reorganization of DNA which leads to highly condensed chromatin stabilized by cross-linked protamine thiols. We provided evidence that snGPx acts as a protamine thiol peroxidase responsible for the disulfide cross-linking and thus necessary to ensure sperm maturation and male fertility. In this survey the main results of several studies on these three selenoproteins will be presented and discussed.

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022.—Overexpression of a Selenoprotein Protects Cells Against Peroxidative Injury and Apoptosis. Rachel Hurst,*+ Yongping Bao,* Gary Williamson,* Witold Korytowski,+ Tamas Kriska+ and Albert W Girotti.+ *Institute of Food Research, Norwich, Norfolk, UK and +Medical College of Wisconsin, Milwaukee, WI, USA.

Selenium has many biological roles that depend on the activity of specific selenium-containing proteins called selenoproteins [1]. One such selenoprotein is phospholipid hydroperoxide glutathione peroxidase (EC 1.11.1.12; Se-PHGPx or glutathione peroxidase type-4) [2]. Se-PHGPx is one of the major defense mechanisms against oxidative damage to phospholipids and cholesterol in biomembranes [3,4]. In this study we have used cell culture models to investigate the effect of increased Se-PHGPx activity and overexpression on cellular protection against protein, lipid and DNA damage. Quantification of carbonyl content, radiolabeled cholesterol oxidation products and DNA damage base products were used as markers for cell damage. Also, fluorescent markers (Annexin-V, ethidium bromide and propidium iodide) and the thiazoyl blue assay were used to investigate apoptosis and cell death. Incubation of cultured hepatocytes with purified Se-PHGPx and a liposome transport complex prior to peroxidative insult with hydrogen peroxide resulted in a decrease in carbonyl content and a reduction in formation of some DNA damage base products [5-hydroxy (OH) methyl (Me) hydantoin, 5-OH hydantoin, 5-OH uracil, 5-OH Me uracil, thymine glycol (cis)] compared with the control (not pretreated with Se-PHGPx). In further studies using a breast carcinoma epithelial cell line, stable overexpression of mitochondrial targeted Se-PHGPx resulted in hyper-resistance to cholesterol hydroperoxide induced injury. Compared with the control cells (minimal or no Se-PHGPx), overexpressing clones were resistant to phosphatidylserine externalisation and fewer cells underwent apoptosis and/or

necrosis following peroxidative challenge. The peroxidation of membrane cholesterol was also significantly reduced in the Se-PHGPx overexpressing cells compared to control cells, as evidenced by decreased formation of radiolabeled cholesterol oxidation products. These results indicate that Se-PHGPx can contribute to cellular protection against apoptosis and oxidative damage to proteins, lipids, and DNA.

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023.—Contrasting Roles of Selenium-Dependent Glutathione Peroxidase-1 (GPX1) in Coping with Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). Xin Gen Lei, Yangxin Fu, James McClung, Xiaomei Zhang and Carol A. Roneker. Department of Animal Science, Cornell University, Ithaca, NY.

Selenium has been widely considered an antioxidant, and GPX1 is the most abundant selenoenzyme in mammals. Our long-term goal is to elucidate the metabolic role of GPX1 and the underlying molecular mechanism. The objective of the present study was to compare impacts of GPX1 knockout on oxidative stress induced by diquat, a ROS-generator, and peroxynitrite (PN), a potent RNS, in mouse hepatocytes and liver. Primary hepatocytes were isolated from GPX1 knockout (KO) or wild-type (WT) mice, and treated with 0.5 mM DQ or 0.1 to 0.8 mM PN for up to 12 h. Time-dependent decreases in cell viability, high levels of apoptotic cells, severe DNA fragmentation, and typical apoptotic signaling were induced by PN in only the WT cells and by diquat in only the KO cells. The PN-mediated protein nitration was much stronger in the WT than the KO cells. To determine if GPX1 potentiated the PN-induced oxidative stress in vivo, we gave KO and WT mice ($n = 3$) an intraperitoneal injection of acetaminophen (300 mg/kg, a PN inducer). At 5 h after the injection, the WT mice showed a much more intense staining against nitrotyosine in the central vein region of liver, and 50-fold higher (822 vs. 16 U/L, $P < 0.01$) plasma alanine transaminase activity than the KO mice. In conclusion, it is most striking that while GPX1 protects against oxidative stress induced by ROS, the enzyme actually provokes oxidative stress induced by PN in vitro and in vivo. These results indicate that antioxidant protection by GPX1 may not be a general property, but depends on the specific nature of oxidants (Supported by a NIH grant DK53018).

024.—The Abnormal Invective Prion Protein (PrPSC) of Ovin Spongiform Encephalopathy (Scrap-pie) Disturbs Copper and Zinc Metabolism and Induces an Oxidative Stress in Neural Cells. Alain Favier. LAN (Laboratory of Nucleic Acid Damage), CEA Grenoble–France.

Recently the membrane located PrP protein. We used mice neural cell lines (GT1–7) non infected, infected (GT-K) and epithelial rabbit kidney cell stably transfected by a doxocyclin-dependent mice PrP gene (A 74). We observe that the expression of PrP is dose dependent to doxocyclin_concentration and induces an increase in copper uptake, as determined by 65 Cu incorporation, and zinc uptake as measured using the fluorochrom zinquin. The overexpression of PrP results also in an increased resistance to metal-induced cytotoxicity. The incubation with PrPSC dramatically decreases copper uptake, Cu-Zn SOD activity and protein level, glutathione reductase and glutathione peroxidase activity, and results in an oxidative stress as observed by MDA. The sensitivity to oxidative stress is also demonstrated by the increase in sensitivity to BSO and syn1-induced apoptosis. Treatment of GT-K cells by congo red normalises copper uptake, antioxidant enzyme activities and cell viability. These results are important to understand the mechanism of neural degeneration in the bovine spongiform encephalopathy and the human new variant of Creuztfeld-Jacob. As other neurodegenerative diseases (ALS, Alzheimer's disease, Parkinson) Creuztfeld-Jacob is an oxidative disease related to an abnormal copper protein.

025.—Female Rats are Protected Against Oxidative Stress During Copper Deficiency. I. Bureau,* E. Gueux,* E. Rock,* A-M. Roussel,[+] A. Mazur* and Y. Rayssiguier.* *Unité Maladies Métaboliques et Micronutriments, INRA-CRNH Saint Genès Champanelle, France and [+]LBSO, Laboratoire de Biologie du Stress Oxydant, Faculté de Pharmacie, UJF, Domaine de la Merci, 38700 La Tronche, France.

Copper deficiency inducing a dramatic decrease of superoxide dismutase activity, leads to alteration of antioxidant defense system. Experiments were conducted in weanling male, intact and ovariectomized female rats, fed either a copper-adequate or copper-deficient diet for 7 weeks, in order to determine whether endogenous estrogen could modulate oxidative stress and the severity of copper-deficiency. Feeding male rats a copper deficient diet induced the typical signs of copper deficiency such as: decreased hepatic copper, growth retardation, anemia, heart hypertrophy, pancreas atrophy, and hypercholesterolemia. Moreover, heart, liver, and pancreas peroxidation in male rats were enhanced by copper deficiency. Although hepatic copper of copper-deficient female was similar to their male counterpart, females were partly protected from the adverse effects of the deficiency (no growth retardation, less severe anemia, lesser extent lipid peroxidation). Thus, female rats are provided with a greater degree of protection against oxidative damages than males. However, females did not appeared protected against pancreas atrophy, heart enlargement, and hypercholesterolemia induced by copper deficiency. The relative protection of female was lost after ovariectomy as shown by decreased body weight and hematocrit, heart enlargement, and higher tissue peroxidation in ovariectomized females compared to intact females. The results suggest that the relative protection of copper deficient female is related to the antioxidant properties of estrogens. The protective action of estrogen against oxidative stress is of particular importance when antioxidant defenses are decreased as shown in this experimental model.

027.—Iron Deficiency and Brain Function. John L. Beard. Department of Nutrition, The Pennsylvania State University, University Park, PA, 16802, USA.

Iron deficiency is associated with alterations in many metabolic processes that may impact brain functioning; among them are mitochondria electron transport, neurotransmitter synthesis and degradation, protein synthesis, organogenesis, and others.

While it is not unreasonable to assume that only very young infants or children are susceptible to the ill effects of iron deficiency, a report published in Lancet in 1996 demonstrated significant alterations in both memory (spatial) and attentional functions in adolescent girls. We have recently determined that young South African post-partum mothers have increased amounts of depression if they are iron deficient; this may be related to slower rates of development in their infants. Studies in iron deficient infants show a delayed nerve conduction velocity than can be explained by both altered neurochemistry and myelination. The recent animal studies of brain iron deficiency during development demonstrate effects on behavior and dopamine metabolism that are only partially reversible. These iron deficient animals show behaviors of decreased exploration, increased anxiety, changes in cocaine addiction and altered movement sensitivity to dopaminergic drugs. We can predict >75% of the variance in some of these behaviors by variations in ventral midbrain iron and DA receptor concentrations. The mechanism of these effects on monoamine metabolism is being explored in cell culture; there is a >50% reduction in uptake of monoamines within 24 hr. of iron chelation. In summary, significant progress in determining neurochemical alterations and the timing of iron deficiency have been made recently, but large gaps in knowledge still exists Supported by PHS-NS35088 and NS34280 and USDA 99-35200-7610.

028.—Zinc and CNS Function. Maureen M. Black. University of Maryland School of Medicine, Baltimore, MD, USA.

The role of zinc in child development is usually assessed by the response to supplementation in populations thought to be zinc deficient. A review of published zinc supplementation trials that examined behavior and development identified 6 trials in infants and toddlers and 3 in school-age children. The two studies that examined activity reported that zinc supplementation was associated with more activity among infants and toddlers. Of the four studies that assessed motor development in infants and toddlers, one found improvements among very low birth weigh infants, one found improvements in the quality of the children's motor development, and two found no impact. Of the four studies that assessed mental development in infants and toddlers, three found no impact of zinc supplement and one found that zinc supplemented children had lower scores than control children. Among school-age children, one study found no impact of zinc supplementation on cognitive performance and two found a beneficial impact. These studies will be summarized together with several recent studies that examine zinc in combination with iron supplementation, and integrated with mechanisms thought to link zinc and CSF function.

029.—Differential Uptake of Aluminum-amino Acid Complexes by Cultured Astrocytes. Aremu, D.A., Tominaga, L. and Meshitsuka, S. Department of Medical Environmentology, Faculty of Medicine, Tottori University, Yonago 683-8503 Japan.

The role of aluminum in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD), continues to be a subject of man's curiosity, more so, when epidemiological studies have been inconclusive and contradictory. Most investigators had also focused on Al complexes, most of which neither naturally exist in biological system nor consumed as such. Thus, the following experiments were carried out in order to determine the relative uptake of Al in complex with some amino acids (aa) including glutamate, glutamine, glycine, and serine using primary culture of cortical astrocytes. Al-citrate complex was also investigated. The cells were exposed to 100_M each of the Al-aa or -citrate complexes for 0.5, 4, 8 or 24 hours. Burst astrocytes served as control to distinguish between mere membrane bound and real Al uptake. Aluminum uptake was measured by atomic absorption spectrophotometry. There was significant real uptake ($p < 0.05$) in Al-ser, Al-gly and Al-gln complexes whereas that of Al-glu was not significant while there was no real uptake in Al-citrate complex. The order of uptake was: Al-ser > Al-gly $>^{*}$ Al-gln $>^{*}$ Al-glu (*sig. diff.). Interestingly, inhibition of Glutamate synthetase (GS) by methionine sulfoximine increased real uptake from Al-glu complex about four times higher than without inhibition. The results show that Al uptake by astrocytes depend on properties of the complexes formed with biological lingands. While the uptake of Al by astrocytes may serve a protective function to neurons, increased CSF levels of serine and glycine even without glutamate and glutamine as in some neuro-pathological conditions can overburden the astrocytes with Al in the forms observed in this study. Conversely, the reduced and none uptake of Al from glutamate and citrate complexes respectively, may aggravate aluminum neural toxicity in vivo. It is probable that GS plays an important role in the transport kinetics of aluminum-glutamate complex.

030.—NMR Studies of the Effects of Aluminum on the Metabolism and Functions of the Cerebellar Astrocytes in Culture. Meshitsuka, S., Hikita, J., Tominaga, L. and Aremu, D.A. Department of Medical Environmentology, Tottori University Faculty of Medicine, 86 Nishimachi Yonago, Tottori 683-8503 Japan.

The neurotoxicity of aluminum is well-known, however, the mechanism has not been clarified yet. Astrocytes play important roles in the central nervous system by maintaining neurons. In order to have more information of the effects of aluminum on the functions of astrocytes, 2D-NMR of DQF-COSY and HSQC were applied to the primary culture systems of cerebellar astrocytes, and granule cell-astrocyte co-culture to establish the substantial interaction between neurons and astrocytes, using 13C labeled glucose as a nutrient. The isotopomers such as citrate, acetate, lactate, pyruvate, alanine and glutamine were observed in the conditioned culture medium of astrocytes. The release of citrate from the astrocytes was specifically inhibited in the presence of aluminum. Citrate was observed inside cells without release into the medium by PCA (perchloic acid) extraction. Citrate may form complexes with aluminum to detoxify the astrocytes.

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031.—Bio-electrochemistry of Selenium Compounds as a Novel Approach Towards Alzheimer's Therapeutics. Gregory I. Giles, Karen M. Tasker, N.M. Giles and Claus Jacob. School of Chemistry University of Exeter, Stocker Road, Exeter, Devon, United Kingdom.

Catalytic agents that mimic the action of the selenium enzyme glutathione peroxidase (GPx) have attracted considerable interest in antioxidant therapy. We have used electrochemical techniques to characterize selenium compounds and demonstrated a high correlation between their oxidation potential and their activity in in vitro GPx assays. Based on these results we have predicted activity in PC12 cell culture indicative of Alzheimer's disease. Cyclic voltammograms of selenium compounds were recorded using a standard Ag/AgCl reference, a Pt counter and a glassy carbon electrode. The value for the first oxidation potential (Epa) ranged from +300 to +1400 mV vs. Ag/AgCl. GPx mimicking activity was established via the catalysis of the reaction of tert-butyl hydrogen peroxide with the thiol protein metallothionein. In PC12 cells amyloid beta peptide (residues 1–40, A_P(1–40)) causes dramatic and selective enhancement of Ca2+ channel activity via a mechanism which involves increased production of oxidizing species. Cells were exposed to A_P(1–40) and varying concentrations of antioxidants and the resulting Ca2+ flux measured by the whole-cell patch clamp technique. The naturally occurring antioxidant melatonin exhibited the least potency (EC50 ~30_M), whereas the commonly used selenium based anti-inflammatory agent ebselen showed increased potency (EC50 ~1_M). In line with the trend in Epa, the most active compound predicted from the GPx assay by far exceeded the activity of ebselen and, astonishingly, was active at nanomolar concentrations (EC50 ~20 nM). This unprecedented antioxidant activity in neuronal cell culture suggests that electrochemical methodology will prove a valuable tool in drug design for Alzheimer's.

032.—Lack of Effect of Micronutrient Supplementation on Cytokine Response to Psychological Stress. Taylor, W., Usher, J.L., Walsh, A., Taxman, B., Salmon, P. and Shenkin A. Departments of Clinical Chemistry and Clinical Psychology, University of Liverpool L69 3GA,UK.

It has been reported that psychological stress may affect immune function via changes in cytokines, the key signaling proteins involved in cellular interactions. Nutritional status also plays an important role in immune status. Many individuals under psychological stress have impaired nutritional status, especially of micronutrients. The effect of nutritional supplementation with vitamins and trace elements on the cytokine responses of students undergoing examinations was investigated. Two groups of medical students were given either a vitamin and trace element supplement ($n = 9$) or a placebo ($n = 13$) for 12 weeks prior to examinations. Cytokine responses were estimated prior to, and following, examinations and at baseline, by stimulating whole blood with Phytohaemagglutinin and Lipopolysaccharide and measuring the amounts of IL-6 and TNF alpha produced. Results are expressed as mean ± sd. In the placebo group, plasma selenium was lower the day before examinations (0.85 ± 0.15 umol/l) than at baseline (1.03 ± 0.13 umol/l; $p < 0.01$), but in the supplemented group there was a significant increase (respectively 1.11 ± 0.14; 0.98 ± 0.16; $p < 0.01$). Plasma zinc concentration did not change in either group. A significant difference in cytokine production was observed between the non-stressed (IL-6: 8.0 ± 2.6 ng/ml. TNF: 1.3 ± 0.4 ng/ml) and the stressed condition. (IL-6: 11.1 ± 3.2 ng/ml. TNF: 2.2 ± 0.6 ng/ml), for IL-6: $p = 0.009$, and for TNF: $p = 0.001$. There was no difference in response between the supplemented and placebo groups. This study confirms that psychological stress affects cytokine production in vitro, but that this is not affected by a micronutrient supplement. Whether subgroups with particular micronutrient status show a different response requires further study.

033.—The Effect of Zinc Supplementation and Psychosocial Stimulation on the Development and Behaviour of Undernourished Jamaican Children. Julie Meeks Gardner,* Christine A. Powell* and Sally M. Grantham-McGregor.[+] *Epidemiology Research Unit, Tropical Medicine Research Institute, University of the West Indies, Mona, Jamaica, W.I. and [+]Centre for International Child Health, Institute of Child Health, London, U.K.

Undernourished children have poor levels of development which continue in later childhood and stimulation improves their development in small studies. Zinc deficiency, common in undernutrition, may also detrimentally affect children's development. We conducted a randomized controlled trial of zinc supplementation and psychosocial stimulation added to the routine care of undernourished children to determine their effects on psychomotor development. The study was a four group factorial design of stimulation alone, zinc supplementation alone, both interventions or routine care only (control). Subjects comprised infants aged 9–30 months who were <-1.5 *z*-scores below the NCHS weight for age references. 114 infants completed the 6 month trial. Cluster sampling was used to assign 18 clinics to stimulation or no stimulation, whereas individual children within each clinic were assigned to zinc or placebo. The stimulation program comprised weekly home visits in which a community health worker demonstrated play and encouraged improved maternal child interactions. The supplementation comprised 10 mg elemental zinc as sulfate in a flavoured syrup or a placebo (syrup only) daily; vitamin and iron drops were provided to all the children. Development (using the Griffiths Mental Development Scales), behavior during the test (using rating scales) and anthropometry (lengths and weights) were measured on baseline and after the 6 month intervention. Home stimulation (using the Bettye Caldwell HOME inventory); mothers' verbal intelligence (using the Peabody Picture Vocabulary test); and the mothers' heights were also measured. There was a main effect of stimulation alone, but not zinc supplementation alone on developmental quotients (DQs). However, there was a significant interaction between the two treatments with children who received both interventions having a significant improvement in developmental quotients and the hand and eye coordination subscale. Zinc supplemented children had fewer episodes of diarrhea, and fewer total days with diarrhea, but their growth did not improve significantly. These results indicate that zinc supplementation along with psychosocial stimulation may provide developmental benefits to undernourished children, with implications for intervention programmes worldwide.

035.—Developmental Consequences of Trace Mineral Deficiencies: Acute and Long-term Effects. Carl L. Keen, Lynn A. Hanna, Louise Lanoue and Michael S. Clegg. Department of Nutrition, University of California, Davis, USA.

Approximately 3% of infants born have at least one serious congenital malformation. In the United States an average of 10 infants per thousand die prior to one year of life and about half of their deaths can be attributed to birth defects, low birth weight, or prematurity. While the causes of developmental abnormalities are multifactorial in nature, the argument will be made that a common factor contributing to the occurrence of developmental abnormalities is sub-optimal mineral nutrition during embryonic and fetal development. Using zinc and copper as examples, evidence will be presented that primary and secondary nutritional deficiencies can rapidly affect the developing conceptus, resulting in gross structural abnormalities. Deficits of zinc and copper can result in rapid changes in cell redox, tissue oxidative stress, inappropriate patterns of cell death, and alteration in cell migration and changes in gene expression. In addition to well recognized structure malformations, mineral deficiencies during perinatal development can also result in behavioral, immunological, and biochemical abnormalities that persist into adulthood. While these persistent defects may in part be attributed to subtle morphological abnormalities, in other cases they may be secondary to epigenic, or developmental changes in DNA methylation patterns. Epigenic defects, combined with subtle morphological abnormalities may influence an individual's risk for certain chronic diseases, and behavioral abnormality, thus increasing their risk for morbidity and mortality later in life.

036.—Molecular and Cellular Aspects of Copper Transport in Developing Mammals. Julian F.B. Mercer. Centre for Cellular & Molecular Biology, Deakin University, Melbourne, Australia.

Copper is critically important for developing mammals, but because of the potential toxicity of copper ions, the supply of copper to the fetus and neonate is carefully regulated by homeostatic mechanisms. Genetic approaches using mutants in humans, mice and yeast, have identified some of the genes involved in copper homeostasis. Copper uptake into cells is mediated by Ctr1, and the essentiality of copper in development of the mouse is demonstrated by the early death of Ctr1 knock out mice (1). There are copper chaperones that function in the intracellular distribution of copper. The Cu-ATPases are important regulators of copper efflux from cells as well as supplying copper to secreted cuproenzymes. Menkes disease in humans, and the mottled mice mutants, are due to mutations of the Menkes gene, ATP7A. Wilson disease and the toxic milk mice are copper toxicosis disorders, due to mutations of the other Cu-ATPase gene, ATP7B. The proteins, ATP7A and ATP7B play many roles in the physiological regulation of copper and are particularly important in the supply and distribution of copper to the fetus and developing neonate. Copper homeostasis involves changes in the intracellular location of the Cu-ATPases. Elevation in intracellular copper induces the movement of these proteins from the transGolgi network to the plasma membrane (ATP7A) or endosomal-like vesicles (ATP7B). We have found that diverse missense mutations prevent the relocalization of the Cu-ATPases in response to copper, and other mutations alter the intracellular localization of the protein. The effects of mutations at a cellular level can explain many features of the genetic copper disorders (2).

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037.—DNA Analysis for Mutations in Cu ATP7B Gene from Indian Childhood Cirrhosis Patients. R. Prasad,* G. Kaur^{+} and B.R. Thapa.** *Department of Biochemistry and **Pediatric Gastroenterology (APC), PGIMER, Chandigarh – 160012, INDIA; $^{+}$Department of Physiology, Chandigarh Medical College, Sector 32, Chandigarh – 160032, INDIA.

Introduction: Indian Childhood Cirrhosis (ICC) is a disorder of copper metabolism which is associated with high copper content which often exceeds the levels observed in Wilson's disease and resulted in swelling and degeneration of liver cell (1). The etiology and pathophysiology is not completely understood. In view of the role of CuATP7B gene in pathogenesis of Wilson's disease, we carried out the study on detection of any defect in CuATP7B gene from ICC patients. Methods: Biochemical parameters were determined as described previously (1). Mutation analysis in CuATP7B gene were carried out as described as Bull et al. (2). Results: Biochemical parameters carried out on ICC patients during admission included serum copper and serum ceruloplasmin which were slightly clevated as compared to controls. Urinary copper in ICC patients was about 21 fold higher than the normal values. Liver biopsies copper was 350 ± 50 mg Cu/g wet tissue. Strikingly, we did not find any mutation in CuATP7B gene from four 1CC patients using either allele specific hybridization or single strand conformational polymorphism analysis. Discussion: The classical form of ICC appears to be associated with the ingestion of boiled milk in copper utensil, high in copper content during early infancy and genetic component has also been proposed (3). Notwithstanding, no mutations were detected after careful analysis of CuATP7B gene. Clearly, this study suggests that a defect in the Wilson disease gene is not responsible for this disorder.

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038.—A Novel Method for Measuring Copper Endogenous Losses Using Stable Isotopes. Jack R. Dainty, Linda J. Harvey and Susan J. Fairweather-Tait. Institute of Food Research, Norwich, UK.

The quantification of mineral absorption and endogenous losses is usually performed using a dual tracer experiment in which oral and intravenous (IV) labels are administered simultaneously. Copper has two stable isotopes (63Cu, 65Cu) and this limits investigations to one labeled tracer; either oral or IV. In previous studies 1,2, only apparent absorption has been calculated from an oral tracer and only endogenous losses from an IV label. A novel approach, developed for the present study, used a labeled oral dose in conjunction with faecal monitoring to calculate both

true absorption and endogenous losses in the same experiment. Twelve healthy, male volunteers aged 32 ± 11 years were recruited as part of a study examining the effect of high, medium and low copper diets on copper absorption and endogenous excretion. At the end of each dietary period, the volunteers were given a drink containing 3 mg of highly enriched 65Cu and 1 mg of holmium for use as a non-absorbed marker. During faecal collection, all the holmium is recovered and any subsequent labeled copper, appearing in the faeces, is assumed to have been absorbed first and then excreted. By fitting an equation to these points, all the labeled copper that has been absorbed and then excreted can be calculated by extrapolation back to the time of dose administration. It was found that an equation of the form $y = mx+c$ fitted the data best. Loss of copper from a single or multi-compartment system would indicate that an exponential equation would be more appropriate but this could not be justified from our data.

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039.—Hyperzincaemia with Hypercalprotectinaemia: a New Disorder of Zinc Metabolism. Identification of Calprotectin (S100A8/S100A9) as the Zinc Binding Protein in Patients with Hyperzincaemia. B. Sampson,*$^{\phi}$ P. Richmond,$^{+\varepsilon}$ B.E. Golden, M.K. Fagerhol,$^{++}$ N. Klein,$^{+}$ I.Z. Kovar,$^{\perp}$ C. Sunderkötter,$^{\perp\perp}$ J.H. Beattie,$^{\alpha}$ B. Wolska-Kusnierz,$^{\beta}$ Y. Saito$^{\chi}$ and J. Roth.$^{\delta}$** *Clinical Chemistry, Charing Cross Hospital, London W6 8RF, UK, $^{+}$Immunobiology, Institute of Child Health, London, UK, **Child Health, University of Aberdeen, Aberdeen, UK, $^{++}$Immunology and Transfusion Medicine, Ullevaal University Hospital, Oslo, Norway, $^{\perp}$Paediatrics, Chelsea & Westminster Hospital, London, UK, $^{\perp\perp}$Dermatology, University of Münster, Germany, $^{\alpha}$Rowett Research Institute, Bucksburn, Aberdeen UK, $^{\beta}$Immunology, The Children's Memorial Health Institute, Warsaw, Poland, $^{\chi}$Pediatrics Tokyo Women's Medical University, Tokyo, Japan, $^{\delta}$Institute of Experimental Dermatology University of Münster, Germany, $^{\varepsilon}$present address: Paediatrics, Children's Hospital Medical Centre, Princess Margaret Hospital for Children, Perth, Western Australia. $^{\phi}$Correspondence to: B Sampson (email: b.sampson@ic.ac.uk).

Calprotectin (complex of S100A8 and S100A9) is the major calcium and zinc -binding protein of phagocytes. We report a new syndrome with recurrent infections, inflammation associated with excessively high plasma concentrations of calprotectin and zinc. Calprotectin in plasma and protein fractions was measured by ELISA assay; Zn by AAS. Plasma proteins were fractionated by size exclusion chromatography and electrophoresis. Mass spectra of purified proteins were determined by MALDI-TOFMS. We present data of 5 patients, 2 of whom are related, with similar biochemical findings of hyperzincaemia (77–200_mol/L, ref. range 11–18_mol/L) and elevated plasma calprotectin concentrations (1.4–9.0 g/L, ref. range <1 mg/L). All patients presented with recurrent infections, hepato-splenomegaly, anemia, and evidence of systemic inflammation. 3 patients had cutaneous inflammation, 2 had thrombocytopenia. 3 patients presented in infancy with severe growth failure. Size exclusion chromatography showed that zinc and calprotectin were associated in a broad fraction with molecular weight range 100–300 kDa. The patients' protein apparently contained normal S100A8 and S100A9 subunits by electrophoresis and mass spectrometry. Dysregulation of zinc metabolism due to accumulation in plasma of S100A8 and S100A9 defines a novel disease entity, which, for the first time, encompasses a pathological role for dysregulation of two members of the large S100 protein family.

040.—Altered Mineral Metabolism in Anencephalic Fetuses? James K. Friel,* Henry Longerich^{+} and Simon E. Jackson.$^{+}$ Departments of Biochemistry*, Pediatrics* and Earth Sciences^{+}, Memorial University of Newfoundland, St. John's, NFLD, Canada, A1B 3X9.

Neural tube defects (NTDs) are congenital abnormalities caused by failure of the neural tube to close during embryogenisis. One NTD is anencephaly (AN) that is a striking abnormality in which there is virtual absence of the forebrain and the skull vault, always resulting in perinatal death. NTDs have a multi-factorial etiology, with Newfoundland having some of the highest rates in the world. The exact mechanism for the defect is unknown and recent success with fortification of flour with folic acid has not eliminated all NTDs. Previous research has suggested that trace elements may play a role. To investigate the role of trace elements, tissues were collected at autopsy from 55 fetuses including 33 AN fetuses and 22 control fetuses (CON). AN fetuses were aborted by either saline or prostaglandin infusion. Control fetuses were term and prater fetuses without congenital defects that died for other reasons. All tissues were collected with parental consent from 3 different regions in Canada. Collections were done on the right side using a titanium scalpel, plastic forceps and all acid-washed materials. Tissues samples included: brain, diaphragm, kidney, liver, lung and sciatic nerve. Tissues and certified reference materials included IEAE muscle and milk powders and NIST liver and oyster powders. Samples were wet-ashed using microwave Parr Digestion Bombs and analyzed using ICP-MS. Values were corrected for wet weight and determined for 18 minerals including: Mg, Ca, Mn, Co, Ni, Cu, Zn, Rb, Sr, Mo, Cd, Fe, Cs, Ba, La, Ce, and Pb. AN fetuses were 27.2 + 7.7 wks gestation, 764 + 863 g birth weight; CON fetuses 32.2 + 11 wks, 1613 + 1218 g birth weight (mean + SD). Liver concentrations (ppm) of Zn (1072 + 322 vs. 666 + 354, $p = 0.001$) were increased in AN, similar to results from a Chinese study, suggesting defective transport of zinc alpha 2-macroglobulin. Cd (ppb) (15 + 26 vs. 54 + 73, $p = 0.007$) and Pb (253 + 324 vs. 568 + 692, $p = 0.027$) were lower in AN liver but showed wide variation in CON tissues. Decreased Ca levels (ppm) in AN liver (232 + 249 vs. 433 + 230, $p = 0.004$) suggest that this may be a key element in the closure of the neural tube. Fe (ppm) in brain (706 + 518 vs. 100 +109, $p = 0.002$), kidney 334 + 202 vs. 217 + 191, $p = 0.038$), and liver (2302 + 1208 vs. 1082 + 932, $p = 0.001$) was higher in AN tissues. Most trace elements are laid down in the last trimester suggesting that higher levels in the younger AN fetuses are due to an altered mineral metabolism. Whether this may cause NTDs or be a result of the disease is unclear. Supported by CIHR, the Janeway Research Foundation and the Spina Bifida Association of Canada.

041.—Identification of a Single Nucleotide Polymorphism in the 3′UTR of Human Glutathione Peroxidase 4. Stéphane Villette,* Janet A.M. Kyle,+ Katrina M. Brown, Fergus Nicol,+ John R. Arthur+ and John E. Hesketh.*** *Department of Biological and Nutritional Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; +Division of Cell Integrity, Rowett Research Institute, Greenburn Road, Bucksburn Aberdeen AB21 9SB Scotland, UK; **University of Aberdeen, Medical School, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK.

Recently, hitherto unrecognized biological roles have been attributed to selenium (Se), particularly in relation to cancer and viral diseases. Se is incorporated in the active site of human selenoproteins as selenocysteine (Sec). Sec is inserted cotranslationally in response to UGA codons within selenoprotein mRNAs in a process requiring a sequence within the 3′ untranslated region (3′UTR), referred to as Sec insertion sequence (SECIS) element. Moreover, structures in the 3′UTR have also been linked with the stability of the message. The glutathione peroxidases, such as phospholipid hydroperoxide glutathione peroxidase (GPX4) are selenoproteins. GPX4 3′UTR was scanned for mutations in a group of 68 healthy Scottish volunteers and we found a novel single nucleotide polymorphism (SNP) by direct sequencing. A T/C variant occurs at position 718 close to the SECIS element. The distribution of that SNP in our population with 34% CC, 25% TT and 41% TC is in Hardy-Weinberg equilibrium. The crucial role of 3′UTR sequences in Sec incorporation suggests that such a polymorphism could affect GPX4 synthesis, especially under conditions of limited Se availability. However lymphocyte GPX4 activity showed a wide inter-individual variation which may mask any potential difference in GPX4 activity between the genotypes. In addition phosphorylation dephosphorylation may also modulate activity so it may be necessary to relate the polymorphism to actual GPX4 protein levels. In parallel to its roles in cell detoxification systems, GPX4 has been proposed to be a key regulator of intracellular events initiating inflammatory responses, being the principal selenoperoxidase involved in the metabolism of 5-lipoxygenase products. A role for GPX4 in leukotriene biosynthesis is supported by our observation of significant differences in 5-lipoxygenase total products between the genotypes, C 718 showing 36% and 44% increases in those products compared to T 718 and T/C 718 respectively. The data also suggest that the SNP718 that we have identified has functional implications.

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043.—The Role of Zinc in Childhood Infectious Diseases. Robert E. Black. Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Zinc deficiency compromises immune function and leads to more frequent and severe infectious diseases; zinc supplementation reverses these adverse effects. Zinc-supplemented children in developing countries have increased lymphocyte counts, especially of helper T cells, and enhanced skin test responses to common antigens, including those in routine childhood vaccines. In addition, they have enhanced responses to experimental vaccines against cholera and rotavirus. In trials assessing prevention of infectious diseases, zinc-supplemented children have less frequent diarrhea, pneumonia, and possibly malaria. Furthermore, a reduction by two-thirds in mortality has been demonstrated with zinc supplementation from one to nine months of age in infants born full-term, but small for gestational age in India. Zinc also has a therapeutic benefit in acute and persistent diarrhea, resulting in a reduction in both the duration and the severity of the illness. A large trial in rural Bangladesh provided oral rehydration therapy to all children with acute diarrhea and a daily (for 14 days) oral zinc supplement to half the children in randomly selected health worker areas. This study found a reduction by half in all deaths, excluding only those from drowning. The role of zinc deficiency in childhood infectious diseases is well established and addressing it by supplementation or food-based strategies will reduce childhood morbidity and mortality.

044.—Genetically Modified Plants for Improved Trace Element Nutrition. Bo Lönnerdal. Department of Nutrition, University of California, Davis, CA.

Deficiencies of iron and zinc are common worldwide. Various strategies have been used to combat these deficiencies including supplementation, food fortification and modification of food preparation/processing methods. A new possible strategy is to use biotechnology to improve trace element nutrition. Genetic engineering can be used in several ways, of which the most obvious one is to increase the trace element content of staple foods, such as cereals and legumes. This may be achieved by introducing genes coding for trace element binding proteins, over-expression of storage proteins already present, and/or by increased expression of proteins responsible for trace element uptake into plants. However, even very high levels of expression may not substantially increase the iron and zinc content unless many atoms of trace elements are bound per protein molecule. Another possibility is to introduce a protein that specifically enhances trace element absorption, even in the presence of naturally occurring inhibitors, thus improving bioavailability. Genetically modifying plants so that their contents of inhibitors of trace element absorption, such as phytate, is substantially reduced is another approach. Low-phytate corn, for example, has been shown to lead to enhanced iron and zinc absorption in humans. Increasing the expression of compounds enhancing trace element absorption, such as ascorbic acid, is also a possibility, although this has received limited attention so far. Iron absorption may be increased by higher ascorbic acid or citric acid contents, but require over-expression of enzymes involved in their synthetic pathways. Finally, a combination of all these approaches, perhaps complemented with conventional breeding techniques, may prove successful.

046.—Zinc Supplementation and Growth of the Fetus and Low Birth Weight Infant. Carlos Castillo-Durán and Gerardo N. Weisstaub. Institute of Nutrition and Food Technology (INTA), Universidad de Chile, Santiago, Chile.

Zinc deficiency limits growth in young children and in animal models also affects fetal growth. However, controlled trials of zinc supplementation during pregnancy in humans have not demonstrated a consistent effect on fetal growth (based on birth

weight and/or duration of gestation) and no effect on post-natal infant growth. Studies that have demonstrated an improvement in some of the variables have been performed in more developed communities; those that have failed to demonstrate a positive effect of zinc on fetal growth, have sampled populations with minimal risk of Zn deficiency or where, besides Zn deficiency, populations are at risk for other nutritional deficiencies, energy, proteins, calcium, iron, vitamin A, with increased intestinal losses and show increased low birthweight rates (between 15% and 45%). Factors that could participate in the differential effects are: age of pregnant women, other nutrient deficiencies, dietary phytates, digestive diseases, energy to zinc ratio, timing for beginning zinc supplementation, compliance. Multiple micronutrient supplementation (including Zn) have not improved fetal growth indices. Few studies of Zn supplementation have been developed in infants born small for gestational age or prematurely. A controlled study (J Pediatr Gastroenterol Nutr 1993) in very low birthweight infants showed a probable positive effect of zinc supplementation on linear growth of girls (D z-score +0.11 vs −0.07) but not in weight. Another study (J Pediatr 1995) of Zn-supplementation for the initial 6 months in small-for-gestational age infants (SGA) demonstrated a significant effect of Zn supplementation on improvement in weight and in length (z-score Zn-suppl −1.28 to −0.66; placebo: -1.43 to −1.47), mainly in girls weaned before 4 months of age, with an additive effect between Zn-supplementation, exclusive breast feeding (>4 mo) and gender; a recent paper (Pediatrics 2001) found an effect of zinc on reducing mortality in SGA infants. We conclude that supplementation trials during pregnancy are not conclusive to indicate zinc supplementation, in spite of experimental evidences that zinc deficiency may retard fetal growth or shorten pregnancy. However, early zinc supplementation in weaned low birthweight or small-for gestational age infants can be effective to improve growth, suggesting prenatal zinc depletion or insufficient zinc intake to support catch up growth.

047.—Zinc Needs During Infancy and Childhood: What Do We Know in 2002? Nancy F. Krebs. Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO, U.S.A.

Dietary zinc requirements of infants depend on the age of the infant, the type of feeding and dietary factors that affect bioavailability, and presence of clinical conditions that may alter absorption as well as losses. Studies of both breastfed and formula fed infants indicate a positive correlation between the amount of absorbed Zn and endogenous fecal Zn, both of which are also positively correlated with the size of the Exchangeable Zn Pool (EZP). The implications of these relationships will be discussed in relation to factors that affect absorption and Zn losses through the gastrointestinal tract. For example, in the older breastfed infant, Zn intake from human milk is quite modest and intake from complementary foods becomes a major factor in total Zn intake. The size of EZP is strongly correlated with dietary Zn intake in 7 mo old breastfed infants. Zn supplementation trials of breastfed infants in the second 6 mo of life have had mixed results, with a positive response in weight gain and linear growth being reported in impoverished populations. This and other factors suggest that increased Zn losses may play a role in the risk of Zn deficiency in infants and children. In premature infants, the mean EZP measured at birth was significantly lower in those born small-for-gestational-age compared to those born with weight appropriate-for-gestational-age. These findings may be relevant to results of Zn supplementation trials of low birth weight infants, which have demonstrated benefits of Zn. Continued advancement in the understanding of the processes of zinc homeostasis in various conditions has direct implications for designing intervention strategies for the prevention and treatment of zinc deficiency.

048.—Zinc Kinetics in Brazilian Women During Pregnancy and Lactation. Ratna Mukherjea,* David M. Shames,* Leslie R. Woodhouse,* Carmina L. Vargas Zapata,+ Carmen M. Donangelo+ and Janet C King.* *USDA Western Human Nutrition Research Center, Univ. of CA, Davis, CA 95616, USA; +Instituto de Quimica, Universidade Federal, Rio de Janeiro, Brazil 21949.

Zinc metabolism is altered during pregnancy and lactation to meet the increased needs of the fetus as well as milk production. There is limited information on the adaptive mechanisms that maintain zinc homeostasis during pregnancy and lactation. The purpose of this study was to investigate longitudinal changes in zinc kinetics during pregnancy and lactation in a group of healthy Brazilian women. Methods: Ten Brazilian women were studied at 12 weeks (early pregnancy: EP) and 35 weeks (late pregnancy: LP) of gestation and at 8 weeks of lactation (early lactation: EL). The women were administered two stable isotopic tracers of zinc at each study period: 1mg orally of zinc tracer highly enriched in 67Zn with breakfast and 0.5 mg IV of zinc tracer highly enriched in 70Zn immediately following breakfast. A baseline and multiple plasma samples were collected over a 24 hour period following infusion of the tracers. Tracer-tracee ratios in plasma were calculated from 67Zn:66Zn and 70Zn:66Zn isotopic ratios measured using ICPMS. Plasma zinc concentrations measured by ICP were corrected for tracer mass. A three-compartment model was developed to describe zinc kinetics in plasma and fitted to the zinc tracer-tracee data using the SAAM II modeling software (SAAM Institute, Seattle, WA). The rate constants and steady state solution of the model for all three time periods were evaluated for significant differences using one way ANOVA. Any p values less than 0.05 were further evaluated using the Bonferroni Multiple Comparison post test with significance between any pairs being assigned significance if $p < 0.05$. Results: Mean dietary zinc in these women was 8.5 mg/day and highly variable. Kinetic analyses indicated that the plasma zinc mass was significantly smaller at EP (1.69 ± 0.27 mg) and EL (1.77 ± 0.28 mg) than at LP (2.08 ± 0.28 mg). The Zn flux between plasma and the most rapidly turning over extravascular pool was higher at LP (8.73 ± 3.53 mg/hr) but not different between EP (5.70 ± 1.73 mg/hr) and EL (5.60 ± 3.05 mg/hr). No other significant changes were found either in the rate constants or steady state measures of the model. Conclusion: The modest changes in zinc kinetics determined from our data in EP, LP and EL were an increase in plasma zinc mass of approximately 20% and an increase in zinc flux between plasma and the most rapidly turning over zinc pool of approximately 50% occurring at LP, the latter probably a reflection of very rapid cell division and protein synthesis in the fetus. The absence of any changes in apparent irreversible zinc loss (urine, feces, integument, milk and flux into very slowly turning over zinc pools) among the three states, despite the increased demands for zinc at LP and EL, suggest compensatory adjustments at the level of the GI tract and kidney and/or zinc flux returning from the very slowly turning over zinc pools.

049.—Fractional Zinc Absorption and Exchangeable Zinc Pool in Women Supplemented with Iron. Manuel Ruz,* Juana Codoceo,* Anabella Rebolledo, Manuel Olivares, Fernando Pizarro, Lei Sian,** Jamie L. Westcott,** Nancy F. Krebs** and K. Michael Hambidge.** *Department of Nutrition Faculty of Medicine, University of Chile, Santiago, Chile [+]Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile, **Section of Nutrition Pediatrics, UCHSC, Denver, CO.

The extent of the potential effects of iron supplementation on zinc absorption and metabolism remains to be elucidated. In this study, we compared the fractional zinc absorption (FAZ) and the size of the rapidly exchangeable zinc pool (EZP) after three months of iron supplementation in non-anemic women consuming ferrous sulfate supplements between meals. Twenty-five apparently healthy women (average age: 26.6 ± 0.7 y) received: 40 mg elemental Fe/d (Group A, n = 7); 80 mg elemental Fe/d (Group B, n = 6); 80 mg elemental Fe/week (Group C, n = 7); and a placebo (Control, n = 5). FAZ was determined on two consecutive days: a) from a single standard meal labeled with 0.8 mg 67Zn; and b) from an aqueous solution labeled with 2 mg 68Zn. FAZ and EZP were determined before iron supplementation began and again three days after completion of the third month of iron supplementation by an extrinsic labeling with enriched zinc stable isotope preparations and a urine enrichment technique. Results are presented as the change at month 3–baseline of selected zinc related variables, adjusted by the variation observed in the Control group (Control = 0). (continued)

	FAZ 67Zn (with meal)	FAZ 68Zn (fasting)	EZP (mg)	Plasma Zn (ug/dL)
Group A	+0.05	+0.10	−10.2	−11.6
Group B	+0.03	+0.02	−16.1	−6.5
Group C	+0.02	+0.17	−11.8	−6.8

None of the differences reached statistical significance, although the trends observed toward increased zinc absorption and decreased EZP and plasma zinc are noteworthy. (Supported by Fondecyt research project 1000896).

050.—Zinc Homeostasis in School-aged Children in Rural Guatemala. Manolo Mazariegos, Brenda Barahona, Raquel Campos, Noel W. Solomons, John Dorsch, Victor Raboy, Jamie Westcott, Sian Lei, Christina Adams, Nancy Krebs and Michael Hambidge. Center for Studies of Sensory, Impairments, Aging and Metabolism (CeSSIAM), 01011, Guatemala City; USDA/ARS National Small Grains Germplasm Research Facility, Aberdeen, 83210, Idaho, USA; Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, 80262, CO, USA.

The objectives of this study are to evaluate zinc homeostasis in children for whom nixtamalized maize is the major food staple and to determine the effects on zinc homeostasis of long-term reduction of the habitually high phytic acid intake. This report covers preliminary metabolic data of the first third of the study population. Twenty children in the community of Buena Vista, Sacatepequez, aged 5–7 years, with weights ranging from 15.7–26.0 kg, were enrolled in year 1 studies. Their families were randomized to receive either lpa-1maize (65% phytic acid reduction); the wild-type isohybrid (control 1) or a locally-purchased Guatemalan maize (control 2) for 3 months. Metabolic studies (including zinc stable isotope tracers and duplicate meals) were undertaken in the village during the final two weeks. The table presents data for: DZ: diet Zn; Ph:Zn: diet phytate: Zn molar ratio; FAZ: fractional absorption Zn; TAZ: total abs. Zn; EFZ: endogenous fecal Zn and UZ: urine Zn.

Variable	n	DZ (mg/d)	Ph:Zn	FAZ	TAZ (mg/d)	EFZ (mg/d)	UZ (mg/d)
Control 1	6	6.1	23:1	0.23 (0.07)	1.32 (0.14)	1.83 (0.46)	0.14 (0.06)
Control	27	9.0	24:1	0.29 (0.06)	2.51 (0.48)	2.03 (0.80)	0.17 (0.07)
Lpa1-1	7	7.2	9:1	0.30 (0.05)	2.19 (0.75)	1.93 (0.90)	0.18 (0.09)

Interim Conclusions: Zinc absorption for control groups was higher than predicted for high phytate: zinc molar ratios. Intestinal excretion of endogenous zinc was relatively high, but was unrelated to phytate intake. These interim data do not indicate any change in zinc bioavailability with moderate reduction of phytic acid intake in this population. Supported by the Thrasher Research Fund, USA and IAEA, Austria.

051.—Copper-deficient Treatment of Mouse Dams Results in Pup Mortality by P13 but the Identical Treatment in Non-pregnant Females Fails to Alter Biochemical Copper Status. Joseph R. Prohaska and Bruce Brokate. Department of Biochemistry & Molecular Biology, University of Minnesota Duluth, School of Medicine, Duluth, MN 55812.

The new US RDA for copper is 900 μg with recommended increases to 1000 μg for pregnancy and 1300 μg for lactation. Copper is an essential transition metal for all biological systems especially during development. Based on our previous rodent models, we questioned if the extra copper increment recommended for pregnancy and lactation was sufficient. We fed female Swiss Webster mice a modified AIN-76A diet low in copper but adequate in all other nutrients (0.3 mg Cu/kg and 43 mg Fe/kg) (−Cu). Half the mice received copper in their drinking water (20 mg Cu/L) (+Cu). In experiment 1 mice were mated to normal males and offered the −Cu or +Cu treatments staring at gestation day 13. Treatments did not affect litter size or pregnancy rate. For 3 litters of +Cu mice 26/26 offspring born were weaned on P21. For 3 litters of −Cu dams 0/26 pups survived beyond P13. The −Cu dams kept on treatment for this 3-week period were killed and compared biochemically to +Cu dams and non-pregnant females, experiment 2, that were kept on the +Cu or −Cu treatment using the exact same diet for 3 weeks. Compared to +Cu dams −Cu dams had 48% lower hematocrits, 89% lower plasma ceruloplasmin activities, 45% lower liver copper levels, and 3-fold higher liver iron levels. The −Cu non-pregnant female mice had no

alterations in any of these copper status indicators compared to +Cu dams or non-pregnant females. Thus, the same copper treatment that fails to support survival during perinatal development has no impact on copper status indicators in adult female mice. Human studies used to establish the copper RDA used adult subjects. We estimate our −Cu mice were receiving 0.05 mg Cu /kg during the 3-week protocol. Though we concede this is severe copper deficiency, these mice were consuming 2.5 times more copper than the current copper US RDA for humans, approximately 0.02 mg Cu/kg. We believe additional studies should be conducted to establish the copper requirement for humans during pregnancy and lactation.

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052.—Metallothionein-leptin Interactions. Beattie, J.H.,* Gordon, M-J.,* Lu, W.,* Trayhurn, P.,+ Giralt, M. and Hidalgo, J.**** *Rowett Research Institute, Aberdeen, UK, +University of Liverpool, Liverpool, UK, **Universidad Autonoma de Barcelona, Barcelona, Spain.

Metallothioneins (MT) have long been associated with heavy metal detoxification and zinc homeostasis[1], and there is now much evidence for their capacity to scavenge free radicals1 and participate in redox reactions[2]. We, and others, have proposed an interaction between the cytokine-like hormone leptin and MT, in which activation of leptin receptors signals upregulation of MT expression[3,4], and lack of MT tends to lead to hyperleptinaemia[5]. Mild obesity has been observed in MT-null mice with a mixed C57BL/6J/129Ola genetic background[5] or backcrossed onto a C57BL/6 background[6], and such observations may indicate a role for MT in regulating appetite and/or energy expenditure. To study the interaction between MT and leptin, we have utilized both MT and leptin deficient mice. MT-null mice on a mixed genetic background (MT-nullm mice) and on a 129/Sv genetic background (MT-nullSv) were fasted for 24 h and the levels of plasma leptin and white adipose tissue (WAT) Ob mRNA were determined. In 3 separate studies, the fasting-induced reduction in ob mRNA and plasma leptin was significantly blunted compared to that in wild-type mice. To determine whether this apparent MT-related influence on ob mRNA and leptin was regulated through the sympathetic nerve system (SNS) activation of white adipocytes during fasting, or through changes in systemic hormones such as insulin, we injected female MT-nullSv mice with either a-methyl-p-tyrosine (aMPT), which blocks noradrenaline release by the SNS, or insulin. Ob mRNA levels in aMPT treated mice were significantly increased in both MT-nullSv and wild-type mice as compared to controls. The response in MT-nullSv mice was marginally blunted. The increase in ob mRNA due to injection of fasted mice with insulin was significantly higher in MT-nullSv mice compared to wild-type animals. These results suggest that MT may have a modulating influence on ob gene expression within white adipocytes. Injection of leptin into leptin-deficient mice stimulated hepatic MT expression and, intriguingly, injection of murine MT into MT-nullm mice transiently suppressed food intake and reduced body weight. In conclusion, MT and leptin show some degree of interdependency and MT may have a modulating effect on mechanisms maintaining energy balance. This work was partly funded by the Scottish Executive Environment and Rural Affairs Department.

054.—Trace Element Uptake and Distribution in Plants. Robin D. Graham. Department of Plant Science, University of Adelaide, Adelaide, South Australia 5005.

There are similarities between mammals and plants in the absorption and transport of trace elements. Mammals may be viewed as an organism wrapped around its food contained within the specialized absorptive organs of the GI tract. The plant on the other hand wraps its food source, the soil, around its specialized absorptive organs, the roots. The chemistry of trace elements in the food in both cases is the thermodynamics of adsorption on charged solid phases embedded in a solution phase of charged ions and heavy metal-binding ligands, together with redox systems in the case of iron and some other elements. Both plants and animals have constitutive absorption systems within the membrane of the cells at the interface with their food that function in nutrient uptake under normal conditions, and inducible, 'turbo' systems that are switched on when the supply of a particular nutrient is less than adequate. These inducible systems are controlled by a feed-back system that senses nutrient status within the organism, and they have fairly high nutrient specificity. In plants, uptake of iron is the most studied of the micronutrients, and this divides the plant kingdom into two groups (or three if we extend the discussion to bacteria). The dicotyledonous plants have a turbo system that is simply an up-regulated version of the constitutive system, consisting of a membrane-bound reductase and an ATP-driven hydrogen ion extrusion pump. In the grasses and cereals, however, while the constitutive system is similar to that of the dicots, the inducible system is remarkably different and highly efficient, utilizing the mugeneic acid class of phytosiderophores (PS). The PS system may in fact be an important port of entry for iron from an iron-rich but exceedingly iron-insoluble planet into the iron-starved biosphere. The evolution of this pathway in the cereals must have become necessary as the planet became increasingly oxygenated during the evolution of life as we now know it. The solubility of iron decreases in the presence of oxygen by factors of 30+ orders of magnitude, but is greatly enhanced at low pH. Thus, iron availability is good in acid soils but these are not favorable to plant growth because of poor availability of other nutrients, notably nitrogen and phosphorus. This conundrum was solved most efficiently by the cereals (and by bacteria that also have inducible systems for iron absorption based on hydroxymate siderophores). Absorption is normally via divalent iron channels after reduction in the membrane, and once absorbed, iron can be stored in both plants and animals as ferritin or transported to active sites by transport-specific organic ligands. The transport of iron and zinc into seeds is dominated by the phloem sap system that has a high pH, like blood, requiring that these heavy metals be strongly chelated. Thus, loading into grains involves three or four genes each that control their chelation, membrane transport and deposition as phytate.

055.—Mineral Bioavailability in Ruminants. Jerry W. Spears. Department of Animal Science, North Carolina State University, Raleigh, 27695-7621.

Efficiency of absorption of many trace minerals and dietary factors that affect bioavailability of minerals differ greatly between ruminants and nonruminants. Ruminant diets are usually high in fiber and considerable digestion of fiber occurs via microbial fermentation in the rumen. However, the quantity of undigested fiber reaching the small intestine of ruminants is much greater than in nonruminants. Association of minerals with fiber fractions in feedstuffs and/or binding of minerals to undigested fiber constituents in the gastrointestinal tract may alter bioavailability of some trace minerals in ruminants. During ruminal fermentation, sulfide and thiomolybdates can be formed that dramatically reduce copper bioavailability. Highly available sources of iron also decrease copper bioavailability. Absorption of selenium from selenite is much lower in ruminants than nonruminants and may relate to reduction of selenite to insoluble forms in the rumen environment. Organic selenium found naturally in plants or in high selenium yeast is more bioavailable than selenium from selenite. High dietary sulfur and the presence of cyanogenetic glycosides in certain legumes have been associated with reduced selenium status. Dietary factors that affect bioavailability of zinc in ruminants are not well defined. Phytate does not affect zinc absorption in ruminants because microbial digestion in the rumen degrades phytate. Limited research suggests that high dietary calcium and phosphorus may reduce manganese absorption. Various supplemental forms of trace minerals also differ in bioavailability.

056.—The Role of Dietary Copper in Osteochondrosis in Pigs. Awatif Aballi* and Dag Austbø.* *Department of Animal Science, Agricultural University of Norway, P.O. Box 5025, N-1432 Ås, Norway.

Osteochondrosis (OC) in pigs is manifested as lesions of joint cartilage. The pathophysiological progression of OC suggests that it occur in growing pigs, although clinical signs may not be evident until adulthood. Dietary Cu plays a role in collagen cross-linking and hence proper growth cartilage structure. Our objectives were to investigate the possibility of elevating hepatic Cu stores in neonates, as well as elevating their Cu intake from milk, through high dietary Cu supplementation (100 mg/kg diet) to their dams during the last trimester of gestation and lactation. Also investigate effects of high ZnO supplementation (2500 mg/kg diet for 15 days) to weanlings of the high and normal dietary Cu (15 mg/kg diet) sows on the pathogenesis of OC in their offspring. Sows fed high Cu diet expressed higher plasma levels of Cu (2.3 mg/L) and Ceruloplasmin (Cp) activity (225 U/L), as well as higher milk levels of Cu at parturition and end of lactation (4.2, 1.6 mg/L respectively) in comparison with sows fed normal Cu diet. Their neonates showed significantly higher hepatic Cu storage at birth, and generally higher plasma levels of Cu and Cp activity, which were assayed at different intervals from birth to slaughter. Those piglets had minor level of knee and elbow OC lesions at 109 kg LBW compared to piglets from sows fed normal Cu. Highest levels of OC lesions were found in offspring of sows fed normal Cu diet, and were supplemented with "pharmacological levels" of ZnO. These pigs expressed generally lower plasma Cu and Cp activity from weaning to slaughter, which is most likely, a response to the profound depressing effect of the high Zn supplementation on Cu metabolism. The pattern of changes in various clinical features assayed in this work suggests that improving the Cu status of the fetus, neonate- suckling play a role in OC expression in pigs later in life, and that "pharmacological levels" of ZnO supplementation to weanlings as growth promoter aggravates the problem of OC. Higher Cu supplementation than currently used in commercial diets of sows during gestation and lactation is recommended.

057.—Effect of Feeding an Organic Polysaccharide Zinc Complex on Growth Performance, Plasma and Fecal Concentrations to Nursery Pigs. Courtney A. Boren and Marcia S. Carlson. University of Missouri, Columbia, MO.

An experiment was conducted to evaluate the effects of feeding titrated concentrations of organic zinc in the form of a polysaccharide complex (SQM-Zn: Quali Tech, Inc., Chaska, MN) on growth performance, plasma zinc and copper concentrations, and fecal zinc and copper excretion of nursery pigs. Three hundred and six crossbred (PIC: C22 X TF4) pigs (avg. 17 ± 2 d of age and 5.14 ± 0.11 kg) were allotted to dietary treatment based on weight and sex. Pigs were housed in an environmentally regulated building with 3 pigs/pen (1/2 × 1.2 m) and 17 pens (replications)/treatment. The experimental diet fed only during Phase 1 utilized six dietary treatments: (1) Basal diet containing 165 ppm Zn as zinc sulfate ($ZnSO_4$), (2) Basal + 125 ppm Zn as SQM-Zn, (3) Basal + 250 ppm Zn as SQM-Zn, (4) Basal + 375 ppm Zn as SQM-Zn, (5) Basal + 500 ppm Zn as SQM-Zn, and (6) Basal + 2,000 ppm Zn as zinc oxide (ZnO). The experimental Phase 1 nursery diet was fed as crumbled pellets from d 1 to 14, a common pelleted Phase 2 was fed d 15 to 28, and a common pelleted Phase 3 diet was fed d 29 to 42. Total lysine concentrations were 1.5% in Phase 1, 1.25% in Phase 2, and 1.1% in Phase 3. All dietary phases contained 165 ppm Zn as $Zn SO_4$, 165 ppm Fe as $FeSO_4$, and 16.5 ppm Cu as $CuSO_4$ from the trace mineral premix. Pigs were bled on d 14 to measure plasma Zn and Cu concentrations. During Phase 1 and Phase 2, grab samples of feces were collected daily and pooled for analysis. During the experiment, there was no difference in ADG, ADFI, or G/F. Pigs fed 2,000 ppm Zn as ZnO had the highest plasma Zn concentrations on d 14 post-weaning ($P > 0.01$) compared with all the other Zn treatments. In Phase 1, pigs fed 2,000 ppm Zn as ZnO had the highest fecal zinc excretion ($P = 0.001$) compared to the other dietary Zn treatments. Plasma and fecal Cu concentrations were not affected by dietary Zn additions during Phase 1 ($P > 0.0001$). These results indicate that feeding lower concentrations of organic Zn, as a polysaccharide complex, may not affect nursery pig performance, but will reduce the amount of Zn excreted.

058.—Comparing Zinc Levels from Brazilian Cattle Tissues Determined by ICP-MS With FAO's and USDA's Releases. Canella,* Grenier,[+] Abdalla,* Heeren[++] and Veado.** *Universidade de São Paulo, Piracicaba, Brazil, [+]Central Service of Analysis du CNRS, Vermaison-Lyon, France, **Scholl of Veterinary Universidade de Minas Gerais, Belo Horizonte, Brazil, [++]Nuclear Engineering Department Universidade de Minas Gerais, Belo Horizonte, Brazil.

Background and Purpose: Appropriately accurate food composition data is a prerequisite for decision making in research and regulatory sciences. FAO and USDA have a long and successful history of producing and disseminating food composition data. Until January 2002, in FAO's Latin Foods, no data for Brazilian meat composition have released. In this work Zn level in bovine tissues (muscle, liver and kidney) from Mexico, USA and Brazil are released. Brazilian data were obtained using the ICP-MS at Central Service of Analysis du CNRS, France. Material and Methods: Cattle specimens from twelve Brazilian cows bos indicus were lyophilized in the LabconcoXXX Freeze Dry System. The ICP-MS used in this study was a PQ 2-Plasma Quad with a Meinhard ultrasonic nebulizer. The measurements were taken in duplicate, using the following operating conditions: ICP-MS power: 1.35 KW; Coolant Argon flow: 14 l/min; Nebulizer Argon flow: 0.8 l/min; Auxiliary Argon flow: 0.8 l/min; Sample uptake rate: 0.6–1 ml/min. The instrument was calibrated with a commercial solutions (SPEX) which contained standards prepared out of 10 ppb multielementary and 1000 ppb Ca solutions. Blanks were also used in order to enable an accuracy of 2–3%. Measurements were carried out using the multi-element modes. An analytical program was established for both calibration and routine analysis. Results: Concentrations bellow are quoted as Zn mg/kg, raw tissues):

Country (Data source)/Tissue	Liver	Kidney	Muscle
Brazil (experimental means)	32.1	15.9	27.0
USA (USDA in www.nal.usda.gov)	39.2	18.5	39.2
Mexico (FAO in www.fao.org)	36	19	39

Conclusion: It should be noted that cattle tissues Zn concentrations are quite variable; they can fluctuate due animal age, sex, status and local water and soil conditions. Most of Brazilian producers do not recognize the Zn deficiency. In fact, effects of a moderate Zn deficiency are harder to recognize and cause significant economic losses through impaired immunity and decreased growth, feed efficiency and fertility. Adequate Zn supplementation should be provided to cattle.

059.—Impact of Pharmacological Zinc and Phytase on Liver Metallothionein Concentration and mRNA Abundance in the Young Pig. Michelle M. Martínez, Gretchen M. Hill, Jane E. Link, Catherine W. Ernst and Nancy E. Raney. Michigan State University, East Lansing, MI, USA.

Pharmacological Zinc (Zn) has been used in developing countries to treat young children with diarrheal disease (Sazawal et al., 1995). In these countries, diets are largely plant based and therefore have an increased phytic acid content. The swine industry uses pharmacological Zn in newly weaned pig diets as an anti-diarrheal agent. Zn absorption is affected by the high phytic acid content in plant-based diets (O'Dell et al., 1972). Evidence suggests that metallothionein (MT), a protein involved in Zn homeostasis, and its mRNA abundance are increased when Zn is supplemented to Zn deficient animals (Shay and Cousins, 1993). Therefore, we hypothesize that diets containing phytase and high Zn will increase hepatic Zn, MT and MT mRNA abundance compared to diets containing only adequate zinc. Twenty-four pigs (5.5 kg) were fed adequate Zn (150 ppm) or one of two pharmacological concentrations (1,000 ppm; 2,000 ppm) as ZnO, with or without (w/o) phytase (0, 500 FTU/kg, Natuphos® BASF). Pigs were killed after 14d of dietary intervention. Liver was excised and frozen for analysis of Zn (atomic absorption spectroscopy) and MT (silver binding assay) concentrations and flash frozen in liquid nitrogen for RNA isolation. Relative MT mRNA abundance was determined by dot blot analysis using a 32P-dCTP-labeled mouse MT-1 probe (Carrasco and Hidalgo, Universitat Atónoma de Barcelona). Pigs fed 1,000 ppm plus phytase or 2,000 ppm w/o phytase had higher hepatic Zn content ($P < 0.02$) than those fed 150 ppm w/o phytase. Hepatic MT was increased ($P < 0.0001$) as dietary Zn increased. The addition of phytase increased ($P < 0.03$) liver MT concentrations. Relative MT mRNA abundance in liver followed a pattern similar to MT protein such that mRNA expression was higher in pigs supplemented with pharmacological concentrations of Zn. MT mRNA expression in pigs fed 1,000 ppm plus phytase was similar to that of pigs fed 2,000 ppm w/o phytase. This study suggests that a pharmacological Zn diet supplemented with phytase enhances hepatic Zn and MT production. It reaffirms the regulatory role of dietary Zn on MT at the transcriptional level and implies that the amount of Zn in oral supplements could be reduced by the addition of phytase.

061.—Iron Regulatory Protein 1: Biosensor of Iron, Oxidative Stress and Nitric Oxide. Richard S. Eisenstein. Dept. of Nutritional Sciences, University of Wisconsin, Madison, WI.

Iron regulatory protein (IRP) 1 and IRP2 are iron-regulated RNA binding proteins that bind to and modulate the use of mRNA encoding proteins required for the uptake (transferrin receptor), storage (ferritin) and use (erythroid 5-aminolevulinate synthase) of iron in vertebrates. In addition to these proteins classically involved in the maintenance of iron homeostasis the tricarboxylic cycle enzyme mitochondrial aconitase (m-acon) also appears to be regulated by IRP suggesting a link between cellular iron status and the metabolic fate of citrate. Up to eight mRNA are targets of IRP action and it is apparent that these mRNA are hierarchically regulated by the IRP (m-acon, 2-fold; ferritin 50-fold). The ability of IRP1 and IRP2 to bind mRNA is regulated through different mechanisms. Formation and loss of an [4Fe-4S] Fe-S cluster in IRP1 modulates its RNA binding activity. In the iron-free form IRP1 binds RNA in a high affinity sequence-specific manner whereas in the [4Fe-4S] state IRP1 fails to bind RNA. Much remains to be understood concerning how changes in cellular iron status are sensed by IRP1 but it is clear that factors that promote removal of the Fe-S cluster, including reactive oxygen species and NO, are important players in this process. In addition, we have shown that RNA binding by IRP is regulated by protein kinase C-dependent phosphorylation thereby providing a pathway for the regulation of iron metabolism by a variety of extracellular agents. We find that phosphomimetic mutants of IRP1 at Ser 138 (S138D and S138E) exhibit a "cluster-instability" phenotype in which the Fe-S cluster is more sensitive to disruption by oxygen and/or oxygen metabolites (20-fold) and NO (15-fold). Furthermore, in rat fibroblasts the phosphomimetic mutants exist primarily in the RNA binding form supporting the hypothesis that phosphor-ylation alters the set-point for iron regulation of IRP1. Taken together, IRP1 is a critical focal point for the regulation of iron metabolism by

iron-dependent and iron independent factors. (Support: NIH/USDA).

062.—Selenoprotein Metabolism and Function. Recent Studies on Functions of Selenoprotein P. Raymond F. Burk and Kristina E. Hill. Division of Gastroenterology and Clinical Nutrition Research Unit, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2279.

Biological functions of selenium in animals are exerted by selenoproteins that contain selenocysteine in their primary structure. Selenocysteine is synthesized and inserted into proteins co-translationally by a complex process. Families of selenoproteins include the glutathione peroxidases, the iodothyronine deiodinases, and the thioredoxin reductases. These are redox enzymes that take advantage of the chemical properties of selenium to catalyze, respectively, removal of hydroperoxides by glutathione, deiodination of thyroid hormones, and support of cellular processes requiring reduction of disulfides. Approximately ten additional selenoproteins have been identified. One of them, selenoprotein P, is an extracellular protein that contains most of the selenium in plasma. Immunohistochemistry demonstrates that it associates with endothelial cells, probably through its heparin-binding properties. Selenoprotein P has been postulated to protect against oxidative injury and to transport selenium from the liver to peripheral tissues. Selenium-dependent protection against diquat-induced liver necrosis in the rat correlates with the presence of selenoprotein P. This supports the oxidant defense hypothesis. Recent results support a transport function. When 75SeO32- is administered to rats by gavage, brain and testis do not begin accumulating 75Se until 75Se-labeled selenoprotein P has appeared in the plasma. Selenium concentrations in those tissues are depressed in selenoprotein P knockout mice. This is evidence that brain and testis depend on selenoprotein P as a source of selenium. Thus, one of the functions of selenoprotein P appears to be to keep brain and testis supplied with selenium. Supported by NIH ES02497.

063.—Regulation of Zinc Metabolism and Functional Outcomes. Robert J. Cousins, Raymond K. Blanchard, Li Cui, Jeffrey A. Bobo, J. Bernadette Moore, Juan P. Liuzzi and Calvert L. Green. Food Science and Human Nutrition Department, University of Florida, Gainesville FL 32611.

Zinc transporters help regulate the supply of zinc essential for growth and regulatory events that influence cellular development and specialized functions. We are investigating the nutritional, hormonal/cytokine and developmental regulation of zinc transporters. ZnT-1 and -2 expression is regulated by dietary zinc in many organs, including small intestine and kidney. ZnT-4 is ubiquitously expressed, but is refractory to zinc intake. ZnT-1, -2, and -4 expression changes markedly during gestation and lactation, with ZnT-2 exhibiting the greatest changes from highly abundant to not detectable. Polyclonal affinity-purified antibodies to synthetic ZnT peptides, western blotting, and immunofluorescence histochemistry were used to measure abundance and define sites of ZnT localization in tissues during changes in zinc intake, pregnancy/lactation and neonatal development. Each ZnT has an endosomal-like appearance in the tissues examined. In the placenta, ZnT-1 and ZnT-4 were concentrated along the villous visceral splanchnopleure, particularly maternally oriented cells. In maternal small intestine, ZnT-1 is apically oriented at the first day of lactation as a line of vesicles between the microvilli and nucleus, but later in lactation it is more widely dispersed. ZnT-4 is also apical, with more at the basolateral membrane later in lactation. ZnT-2 is localized near the microvilli during gestation, but is not expressed after early lactation. ZnT-4 is found in the basal area of cells surrounding the alveolar ducts of the mammary gland. Changes in ZnT-1, -2, and -4 expression indicate these transporters contribute to the maintenance of adequate zinc nutrition for fetal/neonatal development. Upregulation of ZnT-1 and ZnT-2 by dietary zinc strongly implicates these transporters in zinc acquisition and/or storage for subsequent systemic needs. Such regulation places these ZnT genes among the ever growing family of zinc-regulated genes. Differential mRNA display and cDNA array analysis have identified zinc-regulated genes in small intestine, thymus, and monocytes. These include the zinc transporters Zip-1 and Zip-2, uroguanylin, Lck, calreticulin, T cell cytokine receptor, heat shock proteins (Hsp40, 60, and 70), and numerous mitochondrial proteins. The vast majority of the transcriptome is not influenced by dietary zinc intake, high or low. Of the genes that are zinc regulated, most are involved with signal transduction, stress responses and redox, growth and energy utilization. These findings provide a genomic footprint upon which to address both the biological/clinical significance of zinc and new avenues for status assessment. Supported by NIH grants DK31127 and DK52412.

064.—Genetic Defects in Copper Metabolism. Z. Leah Harris. Johns Hopkins University, Baltimore, Maryland.

Genetic defects in copper metabolism highlight the delicate balance mammalian systems have developed to achieve normal copper homeostasis. Menkes disease, the mottled mouse, the Atox-1 deficient mouse, and the ctr-1 knockout mouse reveal the importance during embryogenesis and early development, especially in the central nervous system, for adequate copper intake. The toxicity associated with copper excess as manifest in Wilson disease, the toxic milk mouse, the LEC rat and copper toxicosis in the Bedlington terrier demonstrate the profound cellular susceptibility to copper overload, in particular in the brain and liver. Ceruloplasmin (Cp) contains 95% of the copper found in human plasma and inherited loss of this protein result in diabetes, retinal degeneration and neurodegeneration. Despite normal copper metabolism, aceruloplasminemic patients and the Cp knockout mouse have disturbed iron homeostasis and mild hepatic copper retention. These genetic disorders of copper metabolism provide valuable insight into the mechanisms regulating copper homeostasis and models for us to further dissect the role of this essential metal in health and disease.

065.—Zinc Transporters and Their Roles in Regulating Intracellular and Organismal Zinc Status. D. J. Eide. Department of Nutritional Sciences, 217 Gwynn Hall, University of Missouri-Columbia, MO 65211.

Zinc transporters are required to mediate uptake of dietary zinc in the intestine, its distribution to the cells of other tissues, and

compartmentalization within those cells. Two families of metal ion transporters, the ZIP and CDF families, are of primary importance for eukaryotic zinc metabolism. We have been using the yeast Saccharomyces cerevisiae as a model to understand zinc uptake and compartmentalization in eukaryotic cells. S. cerevisiae has five members of the ZIP family and five CDF family members. Recent studies have made great strides in understanding the function of these proteins. Among the ZIPs, Zrt1 and Zrt2 are plasma membrane transporters responsible for zinc uptake. In contrast, the Zrt3 protein localizes to the vacuole membrane and is responsible for mobilizing stored zinc from this compartment to the cytoplasm under conditions of zinc deficiency. Among the CDF proteins, Zrc1 and Cot1 transport excess zinc into the vacuole when zinc is plentiful and also play important roles in zinc detoxification. The Msc2 protein may transport zinc into the lumen of the endoplasmic reticulum to supply zinc to proteins in the secretory pathway. Thus, members of the ZIP and CDF families play a variety of roles in the utilization of zinc by yeast cells. There are twelve ZIP and seven CDF family members encoded by the human genome. Some of these proteins have already been implicated in zinc transport and it is intriguing to speculate that many will play roles related to those proposed for their yeast relatives.

066.—Functional Studies on the Menkes (MNK) Copper Translocation P-type ATPase in Mammalian Cells. J. Camakaris,* I. Voskoboinik,* C. Lane,* J. Mar,* M. Greenough[+] and M Petris.[+]** *Department of Genetics, University of Melbourne, Victoria, 3010, Australia, [+]Centre for Cellular and Molecular Biology School of Biological and Chemical Sciences, Deakin University, Burwood, Victoria, 3125, Australia, **Department of Nutritional Sciences, University of Missouri, Columbia, Missouri, U.S.A.

Copper is an essential yet potentially highly toxic trace element. Copper homeostatic mechanisms are therefore vital for all organisms. The Menkes (MNK, ATP7A) transmembrane copper-translocating P-type ATPase plays a pivotal role in mammalian copper homeostasis. Mutations in the Menkes genes cause Menkes disease in humans, which is a usually lethal X-linked inherited Cu deficiency disorder. The MNK copper translocating ATPase exhibits Cu regulated vesicular trafficking as well as constitutive recycling between the trans-Golgi network and the plasma membrane. This allows excess Cu to be effluxed. The molecular basis as to why certain mutations in ATP7A are lethal while others result in milder symptoms is not known. Studies on the catalytic mechanism and trafficking of normal and mutant forms of MNK should elucidate how this protein functions in normal copper homeostasis. We have expressed MNK in yeast. These studies have revealed that it is transiently phosphorylated by ATP in a copper-specific and copper dependent manner and appears to undergo transitions in accordance with the classical P-type ATPase model. Furthermore our data supports the notion that putative Cu binding sites at the N-terminal domain of MNK are not essential for catalytic activity but act as Cu sensors and/or Cu scavengers at low Cu concentrations. In order to further investigate the mechanisms of copper-regulated trafficking we have blocked clathrin-mediated endocytosis by transient expression of dominant negative dynamin and eps-15 proteins. These studies indicate that MNK can utilize both clathrin-dependent and clathrin-independent endocytic mechanisms. Current studies are aimed at determining the molecular mechanisms of how Cu regulates the trafficking pathway in various cell types.

067.—Functional and Molecular Responses of Human Intestinal Caco-2 Cells to Copper Treatment. Kathryn A. Bauerly, Shannon L. Kelleher and Bo Lonnerdal. Department of Nutrition, University of California, Davis, CA 95616, USA.

Little is known about the mechanism of copper (Cu) absorption and its regulation. Ctr-1 is a Cu transporter in the apical membrane of intestinal cells thought to transport Cu across the epithelia for absorption. Atp-7a resides in the basolateral membrane and is known to be involved in Cu export from the intestinal cells. The objective of this study was to investigate whether absorption and transepithelial movement of Cu correlated with gene expression of ctr-1 and atp-7a in an experimental model of human absorptive enterocytes. Caco-2 cells were plated onto transwell chambers and allowed to form monolayers. Cells were then treated with 0, 3, 15, 47, or 94 mM Cu supplemented media in the apical chamber for one week. Uptake and transepithelial movement of 67Cu was examined and gene expression of ctr-1 and atp-7a was measured. As Cu supplementation increased, Cu uptake into the cell decreased, while transepithelial movement of Cu across the cell increased. Gene expression of ctr-1 measured by Northern analysis decreased 20%, 56%, 84%, and 76%, respectively from unsupplemented controls. There were no changes in the expression of atp-7a in response to Cu supplementation. This study demonstrates the functional response of Caco-2 cells to Cu treatment and that Cu absorption may be regulated by ctr-1.

068.—Copper Ion Signaling Through Mac1p Phosphorylation in Yeast Copper Homeostasis. Jesse Yonkovich, Xiaoli Shi and Zhiwu Zhu. Department of Environmental Toxicology, University of California, Santa Cruz, CA 95064.

The yeast copper-sensing transcription factor Mac1p has been shown recently to undergo phosphorylation; this modification is required for the Mac1p to bind to the copper ion responsive elements (CuREs) in the CTR1 promoter. Reported disruption of the DNA-bnding by exogenous copper ions suggests that the phosphorylated Mac1p is the sensor for increases in copper ion concentrations. We have identified a threonine residue at position 305 of Mac1p as one of the phophorylation sites. The mutation of the Thr305 to alanine (T305A) in Mac1p affects the phosphorylation state of Mac1p. Unlike the wild type Mac1p however, the DNA-binding by this mutant is not disrupted by exogenous copper ions. That phosphatase treatment halts the mutant DNA-binding suggests that there are other phosphorylation site(s) in Mac1p, and that phosphrylation at those sites is critical for Mac1p to bind to DNA. In cells expressing the T305A Mac1p mutant, the transcriptional inactivation of CTR1 (presumably of CTR3 as well) is defective in response to increases in copper ion concentrations. Furthermore, the cells are highly sensitive to exogenous copper ions and the copper sensitivity is due to CTR1 and CTR3 gene expression. This proves that the control of copper uptake is the primary defense mechanism against copper toxicity. These results indicate that

Mac1p phosphorylation is critical for copper ion signal transfer in yeast copper ion homeostasis.

069.—Copper Induced Proteolysis of the CopZ Copper Chaperone of Enterococcus Hirae. Zen Huat Lu and Marc Solioz. Department of Clinical Pharmacology, University of Berne, 3010 Berne, Switzerland.

The cop operon is a key element of copper homeostasis in Enterococcus hirae. It encodes two copper ATPases, CopA and CopB, the CopY repressor, and the CopZ metallochaperone. It was previously shown that transcription of the operon is induced by copper. The concomitant increase in the levels of Cop proteins, particularly the CopB copper export ATPase, allows uncompromised growth of E. hirae in up to 5 mM ambient copper. We here show by Western blotting that the steady-state level of CopZ was increased only up to 0.5 mM copper. At higher copper concentrations, the level of CopZ was decreased and became undetec-table at 5 mM media copper. When CopZ was over-expressed from a plasmid, the cells exhibited increased sensitivity to copper and oxidative stress, suggesting that high CopZ expression could become toxic to cells. In wild-type cells, the level of mRNA transcripts from the cop operon remained high in up to 5 mM copper, suggesting that CopZ was proteolyzed. Cell extracts were found to contain a copper activated proteolytic activity that degraded CopZ in vitro. In this assay, Cu-CopZ was more susceptible to degradation than apo-CopZ. Growth of E. hirae in copper increased the copper inducible proteolytic activity in extracts. Zymo-graphic studies showed the presence of a copper dependent proteases in crude cell lysates. Thus, copper-stimulated proteolysis plays an important role in the regulation of copper homeostasis in E. hirae.

070.—Copper Repletion Upregulates Iron Transport and Expression of Iron Transport Factor Genes in Intestinal Cells. Okhee Han* and Marianne Wessling-Resnick.[§] *Nutritional Sciences, Oklahoma State University, Stillwater, OK 74078 and [§]Department of Nutrition, Harvard School of Public Health, Boston, MA 02115.

The connection between Cu and intestinal Fe absorption is well known but it is not clear how Cu is involved in the process(es) of intestinal iron absorption. To elucidate how Cu influences intestinal Fe transport, fully differentiated Caco-2 cells grown on microporous membrane inserts were supplemented with 1_M CuCl2 for 7–8 days. Cu-treated cells had 10-fold higher levels of cellular Cu compared to control. Cu-repleted cells stimulated apical 55Fe uptake and transepithelial 55Fe transport across cell monolayers. Northern blot analysis showed that expression of apical metal transporter-1 (DMT1), the basolateral Fe transporter-1 (Fpn1) and the putative feroxidase hephaestin (Heph) was increased by Cu repletion and desferrioxamine, iron chelator treatment. These results suggest that upregulated DMT1, Fpn1 and Heph expression may be involved in Cu-mediated Fe transport process(es). An apical membrane ferrireductase activity was also increased by Cu supplementation, indicating that its function may contribute to the enhanced Fe transport in Cu-replete cells. These results therefore suggest that Cu supplementation may promote Fe depletion by stimulating Fe efflux, thereby creating a Fe deficiency state to upregulate Fe transport factors.

072.—Risk Assessment of Essential Trace Elements: an International Perspective. James R. Coughlin. Coughlin & Associates, Newport Coast, CA.

There has been a great deal of progress achieved by both toxicologists and nutritionists worldwide on establishing and refining methodologies for conducting improved risk assessments for the essential trace elements (ETEs). The specific goals of these activities have been to better define the shape of the ETEs' U-shaped dose-response curves and to set upper safe intake levels for these nutrients. Nutritionists have faced the challenge of incorporating concepts beyond the simple alleviation of nutrient deficiencies to include strategies to reduce the risks of chronic disease, and this can sometimes mean setting a higher recommended dietary allowance for an ETE. An additional challenge occurs when the recommendation for higher intake of an ETE to prevent chronic disease may lead to the possibility of increased toxicity, often at another organ site unrelated to the essential function(s) of the nutrient. For ETEs, the U.S. and other national and international agencies have recently begun to use the well-known techniques of chemical risk assessment, including adjustments for homeostatic, pharmacokinetic, pharmacodynamic, bioavailability, speciation and essentiality factors, in establishing upper safe intake levels. The risk assessment methodologies of four international bodies will be compared and contrasted: a National Academy of Sciences' Food and Nutrition Board Subcommittee, the European Union's Scientific Committee on Food, the International Program on Chemical Safety, and the UK's Expert Group on Vitamins and Minerals. This groundbreaking activity represents the coming together of experts in nutrition, toxicology, epidemiology, clinical medicine and quantitative risk assessment to develop innovative risk evaluation methodologies that will serve to ensure safe intake levels of ETEs.

073.—Arsenic (As) in the Environment: Health Effects and Risk Evaluation. Charles O. Abernathy. Office of Science and Technology, US EPA, Washington, DC.

The acute toxicity of arsenic was known to the ancients; it was used as a poison in many cultures. However, from the environmental standpoint, the greatest interest is in the effects of small chronic doses of As which are often difficult to detect and record. These doses are of interest since people are exposed to As through air, soil, water and food. In 1968, a report from Taiwan linked As exposure from drinking water to skin cancer. Other reports followed and chronic low-level As exposure has been associated with many cancers (skin, bladder and lung) and non-carcinogenic effects (dermal effects, cardio- and cerebrovascular diseases and diabetes). While working on a new As Maximum Contaminant Level (MCL), the US EPA contracted two As reports by the National Research Council (NRC). The 1999 one focused on bladder cancer and derived a 1% effective dose (ED01) of about 400 μg/L. As this level was only 8-fold above the Interim MCL of 50 μg/L, they recommended lowering the MCL. The 2001 report looked at bladder and lung cancer and stated that the risks might be higher than previously anticipated. Recent work showed that the trivalent methylated metabolites of As were more toxic than arsenite (As+3) and could react directly with DNA. The NRC recommended using a linear extrapolation from the calculated ED01. On Oct. 31, 2001, EPA announced a MCL of 10 μg/L. (These opinions are those of the author and not necessarily those of the EPA.)

074.—Studies of Prenatal Exposures to Methyl Mercury in Fish. Tom Clarkson. Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY.

Our studies of the outbreak of methyl mercury poisoning that took place in rural Iraq in the fall and winter of 1971–72 raised the possibility that prenatal exposure to methyl mercury experienced by high fish consumers might result in damage to the developing fetal brain. The exposure to methyl mercury in Iraq was from home made bread contaminated with a methyl mercury fungicide. An opportunity was found in the Seychelles islands to study a population having a high dietary intake of ocean fish. This would allow us to check if estimates of prenatal risk from a subsistence desert population were valid in a well-nourished population consuming methyl mercury naturally bio-accumulated in ocean fish. Two cohorts, each of over 700 of infant mother pairs, were established, the first acting as a pilot to the second main study. Prenatal exposure was measured as the average maternal hair levels during pregnancy. Developmental and neuropsychological tests have been made longitudinally on the offspring from ages 6 to 107 months. Overall, evidence of adverse effects from methyl mercury has not yet been detected. The outcome of this study will be compared with other ongoing and previous studies of prenatal exposure to methyl mercury.

075.—Confirmation of Safe Level of Zinc Intake as Assessed by Immune Function and Copper Status in Healthy Adult Men. Maxine Bonham,* Jacqueline M. O'Connor,* James Coulter,* H. Denis Alexander,[+] C. Stephen Downes,* Bernadette M. Hannigan* and J.J. Strain.* *Northern Ireland Centre for Food and Health (NICHE), Department of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland. and [+]Department of Haematology, Belfast City Hospital, N. Ireland.

Recent guidelines [1] have established a lowest-observed-adverse-affect-level (LOAEL) of total zinc intake at a dose of 60mg/day and extrapolated from this an upper level (UL) of safe zinc intakes. The aim of this trial was to examine the effects of zinc supplementation (to include dietary intakes) at the UL of 40 mg/day on immune function and copper status. Thirty eight subjects were recruited onto a double-blind placebo controlled intervention trial. One group (n = 19) took zinc supplements (30 mg/day) for 14 weeks followed by copper supplements (3 mg/day) to alleviate side effects, if any, from the zinc supplements upon body copper status. A second group (n = 19) took placebo only for the duration of the trial. Blood samples were taken at baseline and weeks 2, 14, 16, 18 and 22 and assessed for full blood profiles, flow cytometric analysis of lymphocyte subsets and a number of putative indices of copper and zinc status. Dietary intakes of zinc were assessed by 4 day dietary records and approximated 9.5 mg/day. Results indicated no effect of zinc supplementation on circulating absolute levels of peripheral blood leukocytes or a number of lymphocyte subsets. Markers of zinc and copper status were also unaffected by zinc supplementation. Independent of supplement, there appeared to be seasonal variations in selected lymphocyte subsets in the group as a whole (n = 38). Significant alterations in absolute levels of B cells ($p = 0.000$), memory T cells ($p = 0.000$) and the adhesion molecule ICAM-1 ($p = 0.000$) were observed. Absolute levels of basophils, basophils, eosinophils, neutrophils, T cells, helper-T, cytotoxic-T, and naïve-T cells, natural killer cells, HLA-DR, the interleukin-2 receptor and the adhesion molecule LFA-1 were unaffected. Findings indicate no adverse effects of zinc intakes of 40 mg/day (30 mg/d supplement and 10 mg/d dietary intake) on immune function or copper status and support the upper level of zinc tolerance established at 40 mg/day. The seasonal variations observed in lymphocyte subsets (whole group) could have implications for the prevalence of seasonal variability in the incidence of infectious diseases.

Funded by the Food Standards Agency (AN0553).

1. Institute of Medicine, Food and Nutrition Board (2001) Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press. Washington D.C.

076.—Uptake, Biotransformation, Accumulation and Excretion of Arsenic by Ruminants Chronically Exposed to Organoarsenicals. H.R. Hansen, A. Raab and J. Feldmann. Environmental Analytical Chemistry, Department of Chemistry, University of Aberdeen, Meston Walk, AB24 3UE, UK.

The population of sheep on North Ronaldsay, a small island north of Scotland, is restricted to life on the beach shore and is chronically exposed to arsenic. The arsenic consumed by the sheep is in the form of organoarsenicals, which is in their main diet—seaweed. Here we evaluate the metabolism of arsenosugars by this rare breed of sheep, which is likely to have the highest arsenic intake of any known mammal in the world. We studied the behavior of the wild sheep and conducted a feeding trial where 12 North Ronaldsay ewes were fed seaweed, and their urine and faeces were collected twice a day. Arsenic concentrations were measured by ICP-MS (for total) and cation and anion exchange chromatography was coupled to ICP-MS to separate and detect the different arsenic metabolites. We estimate a daily arsenic intake by the sheep at up to 35 mg, which varies with weather, tidal cycle and season. The main food source for the sheep are the brown macroalgae Laminaria spp (74 mg As/g dry mass, present mainly as arsenosugars) but the sheep was observed to feed on 16 different species of seaweed. The urinary arsenic concentration in the current study was up to 1000 times higher (21.7 ± 9.9 mg As L-1 (n = 54)) than for non-exposed sheep. The main urinary arsenic metabolite excreted was dimethylarsinic acid (DMA(V))—the very same main metabolite that inorganic arsenic is metabolised into by most mammals. Besides traces of other methylated arsenic compounds, we found 7 unknown urinary arsenic metabolites. From a previous study, we know that arsenic accumulates in wool and tissues, mainly in the fatty tissues. The concentrations in the tissues were elevated but below the safety limit for food in the UK (1 mg kg^{-1}) and are not a matter for concern. Despite the high arsenic intake the sheep show no abnormality or signs of toxicity with tallies well with the general belief that arsenosugars are non-toxic. However, it is of special interest that the metabolism of "non-toxic" arsenosugars has similarities to the metabolism of "toxic" inorganic arsenic—and we would stress that as long as the metabolic pathway is not fully understood, further study into the arsenosugar metabolism remains important.

1. Feldmann, J., Balger, T., Hansen, H. and Pengpreecha, P. (2001) An appetite for arsenic—the seaweed-eating sheep from Orkney (Scotland), RSC proceedings, Durham 2000.

077.—Copper-induced Hepatotoxicosis with Fibrosis in Artificially Fed North Ronaldsay Lambs: a Paradigm for Susceptible Infants Exposed to Excess Copper in Drinking Water. Susan Haywood,* A.M. Mackenzie, T. Müller, Claire L. Williams and M. Loughran. *Department of Veterinary Pathology, University of Liverpool, UK. +Harper Adams University College, Shropshire, UK.; Department of Paediatrics, University of Innsbruck, Austria.

Idiopathic copper toxicosis (ICT) and endemic Tyrolean infantile cirrhosis (ETIC) are liver Cu overload syndromes of childhood associated with excess Cu intake and genetic predilection. These diseases most commonly occur in bottle-fed infants with a higher than recommended Cu content of 3–26 mg/l (WHO guidelines for Cu in drinking water <2 mg/l Cu). North Ronaldsay sheep have been proposed as animal models of this childhood disease (Haywood et al, 2001). This study attempted to reproduce the disease in young North Ronaldsay lambs, Ronaldsay Cu toxicosis (RCT), simulating the conditions found in affected infants. Methods: Thirty lambs were assigned at 17 days of age to the trial. Four lambs were killed initially and 24 were randomly assigned to the following groups: 8 lambs to Group1, which were artificially reared on a formula diet (Shepherdess, SCA Nutrition, containing 0.9 mg Cu/kg DM). Eight lambs were fed the milk replacer with the addition of 5 mg/l Cu (Group 2) and 8 with 10 mg/l Cu (Group 3). Four from each treatment group were killed at 57 and 86 days old (A&B respectively). Liver samples were fixed in 10% formalin. Paraffin-embedded sections were stained with HE, with rhodanine for Cu, Massons trichrome for collagen; also immunostaining for_smooth muscle actin (ASMA). Cu analysis was performed by ICP-MS on dried liver samples. Results: Copper analysis: Liver Cu was maintained at <300_g/g in the control groups whereas Cu rose to >1400_g/g in all trial groups, declining terminally in group 3B. Histopathology: Groups 2 and 3A showed marked apoptosis, nuclear anisoploidy and mitoses in viable hepatocytes. Copper accumulated histochemically in hepatocytes and Kupffer cells. In groups 2 and 3 B, karyomegaly was more pronounced and mitotic activity declined. Marked cytoplasmic vacuolation with bile pigment was present in hepatocytes. Fibroplasia was marked with portal-portal bridging and florid pericellular fibrosis. Marked Ito cell activation was associated with collagen synthesis. Histochemical copper disappeared terminally from hepatocytes but was retained in Kupffer cells. Conclusion: Copper-induced hepatotoxicosis with marked pericellular fibrosis has been induced in artificially fed lambs with milk containing 5–10 mg/l Cu-a response very similar to that evoked in susceptible infants within this dietary Cu range. Although it remains to be established whether RCT is the genetic equivalent of the non-Wilsonian infant disorder, North Ronaldsay sheep are a useful model of a low threshold Cu sensitivity during the suckling period and for setting the safety limits for Cu in formula milk.

1. S Haywood, T Müller,W, Müller et al. (2001) Copper-associated liver disease in North Ronaldsay sheep: a possible animal model for non-Wilsonian hepatic copper toxicosis of infancy and childhood. J Pathol 195: 264–269.

078.—Advances in Risk Assessment of Trace Elements: Boron as a Case Study. F. Jay Murray. Murray & Associates, San Jose, CA.

The trace element boron (B) is ubiquitous in the environment. The primary sources of dietary exposure to B are fruits, vegetables, and nuts; the mean dietary consumption in the United States is slightly greater than 1 mg B/day. In recent years, risk assessments of boric acid (BA) have been performed by several well-respected organizations, including IEHR, ECETOC, IPCS, WHO, and the NAS Food and Nutrition Board. Although the final results of these risk assessments are similar, the methods and approaches differed. In each of these risk assessments, the pivotal study was a developmental toxicity study in rats with a no-observed-adverse-effect level (NOAEL) of approximately 10 mg B/kg/day. These risk assessments employed varying uncertainty factors (UF) in the range of 25–60, yielding estimates of tolerable daily intake levels in the range of about 10–25 mg B/day. A limitation of previous risk assessments was the absence of specific data on the renal clearance of B in pregnant rats and pregnant women. New data has demonstrated that when renal clearance was normalized to body weight (ml/min/kg), pregnant rats cleared B (administered as BA) at a rate roughly 3 times greater than pregnant women. In addition, the renal clearance of B among pregnant women varied by a factor of about 2. These new data support the use of reduced UF. Total UF in the range of 22–44 are scientifically justified for B. An acceptable daily intake of 14–27 mg B/day may be estimated by applying a total UF of 22–44 to the NOAEL of 10 mg B/kg/day. The use of data-adjusted UF should lead to better estimates of risk. Importantly, dietary consumption of B in the United States is well below all of the estimated.

079.—Micronutrient/Metabolite Intake and Metabolic Harmony. Bruce N. Ames. University of California, Berkeley/CHORI, 5700 M.L. King, Jr. Way Oakland, CA 94609.

Maximum health and life span require metabolic harmony. An optimum intake of micronutrients and metabolites, which varies with age and genetic constitution, would tune-up metabolism and give a marked increase in health at little cost. It is inexcusable that anyone in the world is deficient for a vitamin or mineral, at great cost to health, when insurance, a daily multivitamin/mineral pill, costs less than $10/yr. The requirements of the old for vitamins/metabolites differ from that of the young. 1) DNA Damage & Metabolism. Inadequate intake of folic acid causes millions of uracils to be incorporated into the DNA of each cell, with associated chromosome breaks: a radiation mimic. Deficiencies of the metabolically connected vitamins B6 and B12 also appear to cause uracil incorporation and chromosome breaks. Folate deficiency is associated with several types of cancer and sperm damage. About 10% of Americans had inadequate folate intake before the recent food fortification, about 10% is low in B6 and about 14% of the elderly is low in B12. Inadequate iron intake (2 billion women in the world; 19% of U.S. menstruating women) causes oxidants to leak from mitochondria and damages mitochondria and mtDNA. Zinc deficiency (about 20% in the U.S.) causes oxidation and DNA damage in human cells. The poor are particularly prone to deficiencies. Ames, B.N., DNA Damage from micronutrient deficiencies is likely to be a major cause of cancer. Mutat. Res., 2001. 475: 7–20. 2) The Km concept & Metabolism. About 50 different human genetic diseases due to a poorer binding affinity (Km) of the mutant enzyme for its coenzyme, can be remedied by feeding high dose B vitamins, which raise levels of the corresponding coenzyme; many polymorphisms also result in a lowered affinity of enzyme for

coenzyme. The Km concept will be relevant for mitochondrial aging as well as for human nutrition. Ames, B.N., I. Elson-Schwab, & E. Silver, High-dose vitamins stimulate variant enzymes with decreased coenzyme-binding affinity (increased Km): relevance to genetic disease and polymorphisms. Am J Clin Nut., April, 2002.

081.—Ferritin, at the Crossroads of Iron and Oxygen Metabolism. Elizabeth C. Theil. CHORI (Children's Hospital Oakland Research Institute), Oakland, CA, USA.

Iron and oxygen are central to terrestrial life. In the physiological environment aqueous iron and oxygen chemistry will produce a ferric ion, billions of times less soluble than cell concentrations, plus radical forms of oxygen that are toxic. Many proteins have evolved to transport iron or modulate the redox chemistry of iron that transforms oxygen in useful biochemical reactions. Only one protein, ferritin, has evolved to concentrate the iron to the levels needed by aerobic metabolism, through the reversible formation of a solid, nanomineral of hydrated, iron-oxide. Predictably ferritin occurs in almost all organisms. The mineral in animals is microcrystalline but in plants is amorphous and high in phosphate, because of protein-targeting to the phosphate-rich plastid. Ferritin is a large, multifunctional, multi-subunit protein with 8 Fe transport pores, 12 mineral nucleation sites facing the internal nanocavity, and 24 sites to create the first mineral precursors from ferrous iron and oxygen, ferric dimers linked by oxo/hydroxo bridges. (Ferritin is thus a potential oxygen trap in anerobes). Regulation of ferritin synthesis in animals uses both DNA and mRNA controls and two genes encoding related subunits, with catalytically active (H) and inactive (L) sites, that coassemble in varying H/L ratios. Ferritin catalysis produces H2O2, making Fe-induced changes in H/L ratios (and catalase/peroxidase expression?) a regulatory response at the Fe/O2 crossroads. The use of ferritin by seeds and developing animals as an iron source led to the demonstration of ferritin as an available dietary iron source and suggest novel potential strategies for managing global iron deficiency.

082.—Iron and copper Interactions in Development and the Effect on Pregnancy Outcome. Lorraine Gambling, Kaila Srai,[+] Susan Dunford, Cedric Fosset, Ruth Danzeisen and Harry McArdle. Rowett Research Institute, Aberdeen UK and [+]Royal Free Hospital, London UK.

Over the past few years, data are beginning to accumulate explaining the interaction between Cu and Fe. Most of the information has been accumulated in cells in culture, mammalian and yeast. Less is known about interactions in whole animals, especially under conditions of growth and development. We have been studying Fe metabolism in pregnancy and in this presentation will present data on the effects of Fe status on expression of proteins of Fe and Cu metabolism and on the pre- and post-natal development of the offspring. Fe deficiency in the mother is reflected in deficiency in the fetus, but to a significantly lesser extent. The deficiency is ameliorated by increased expression of several proteins of Fe metabolism in the placenta. Transferrin receptor, IRE-regulated DMT1 and the Cu oxidase involved in efflux all increase expression. In contrast, non-IRE-regulated DMT1 and IREG1 do not change, at least at mRNA level. Cu levels in maternal tissues and placenta increase, but decrease in the fetal liver. There is no significant change in expression in the proteins of Cu metabolism. Fe deficiency results in smaller fetuses, and the decrease in size is also found in newborn pups, who also have larger hearts, smaller kidneys and spleens than their normal counterparts. Despite normal Fe intake throughout their lives, these pups have increased blood pressure, even at 16 weeks of age.

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083.—Hephaestin and Ireg1 mRNA and Protein are Regulated by Iron Status in Mouse Duodenal Enterocytes. Huijin Chen,[‡] Trent Su,[‡] Zouhair Attieh,[‡] Steve Zippin,[‡] Greg Anderson[§] and Chris Vulpe.[‡] [‡]Department of Nutritional Science and Toxicology, University of California, Berkeley [§]Joint Clinical Sciences Program, Queensland Institute of Medical Research and University of Queensland, PO Royal Brisbane Hospital, Brisbane Queensland 4029, Australia.

Hephaestin (Hp) is a membrane-bound multicopper ferroxidase necessary for iron egress from intestinal enterocytes into the circulation. We investigated how iron status regulates Hp and Ireg1 mRNA expression, protein levels and Hp activity. Experiments were conducted on C57BL/6J control mice subjected to three separate dietary iron treatments and on the sex-linked anemia (sla) mouse, which has a block in intestinal iron transport. For dietary experiments over a 6-month period, C57BL/6J mice were fed iron deficient (2–3 ppm iron), iron overload (2% carbonyl iron) or control (40–50 ppm iron) diet, and sla mice were fed control diet. The sla mice were functionally iron deficient: Both sla and iron deficient C57BL/6J mice had a decrease in non-heme iron in the liver. In duodenal enterocytes, Northern blot analysis showed that mRNA levels of both Hp and Ireg1 increased. At the protein level, Hp consistently increased two-fold and Ireg1 increased dramatically as shown by Western blots. Furthermore, Hp oxidase activity showed a similar pattern using native in-gel PPD oxidase assays. The sla mice, despite exhibiting these iron deficiency responses, had ferritin protein levels that were the same as in iron sufficient control mouse enterocytes. Thus, while Hp and Ireg1 mRNA, protein levels and activity are regulated by iron status in the duodenum of mice, ferritin is not. We hypothesize that Hp and Ireg1 levels are increased in iron deficient and sla mice to stimulate increased iron egress from intestinal enterocytes for absorption, but in sla mice the iron cannot be released. In support of this, sla mice accumulate excess Fe in intestinal enterocytes vs controls. It is considered that sla mouse has a mutant of Hp, so it could block iron export from intestinal enterocytes. The excess iron in sla is likely not bound by ferritin but may be instead in an intracellular compartment. In vivo Fe staining patterns, now underway, may provide clues as to the difference between iron overloaded control mice and sla mice.

084.—Effect of Maternal Iron Status on Mammary Gland Iron Transporters in the Rat. Weng-In Leong and Bo Lönnerdal. University of California, Davis, Davis, CA, USA.

Little is known about the mechanisms of iron transfer from plasma to milk. Recently, DMT1 and FPN1, were identified as iron transporters in the small intestine. Whether these transporters are present in the mammary gland, and are involved in iron transfer from plasma to milk is not known. In this study, we examined the mRNA and protein expression of these transporters in rat mammary gland at day 1, 5, 10 and 20 of lactation, and the effect of iron deficiency on their expression. Rats were fed either control diet (35 mg Fe/kg diet) or low Fe diet (8 mg Fe/kg diet) 3 weeks preconception through lactation. Liver Fe and hemoglobin increased during lactation. Mammary gland Fe remained constant, while milk Fe decreased during lactation. Relative DMT1 and FPN1 mRNA expression decreased during lactation. The low Fe group had lower liver Fe at d10 and 20 of lactation, but there was no difference in milk Fe. Mammary gland Fe was lower in the low Fe group at d10, but unchanged at d20. DMT1 mRNA expression was unchanged at d10, but protein expression was 5-fold increased in the low Fe group. At d20, DMT1 mRNA expression was increased 2-fold in the low Fe group, but protein expression was unchanged. FPN1 mRNA and protein expression was unchanged at d10. At d20, FPN mRNA expression was increased 2-fold, whereas protein expression was decreased 40% in the low Fe group.

These results show that both DMT1 and FPN1 are present in higher amounts during early lactation, and possibly are involved in the transfer of iron from plasma to milk, thereby playing a role in maintaining milk Fe secretion during iron deficiency.

085.—Spatial and Temporal Expression Patterns of Selenoproteins During Embryogenesis in Zebrafish. Alain Lescure,* Gregory V. Kryukov,[+] Vadim N. Gladyshev,[+] Christine Thisse, Alain Krol1 and Bernard Thisse,**** *UPR 9002 du CNRS, Institut de Biologie Moleculaire et Cellulaire, Strasbourg (France), [+]Department of Biochemistry, University of Nebraska, Lincoln (Nebraska, USA), **Institut de Genetique et Biologie Moléculaire et Cellulaire, Illkirch (France).

Over the last decade, the zebrafish embryo has become one of the major model organisms for the study of vertebrate embryogenesis. Its transparency is one of its main assets, allowing one to view the details of embryological processes, which take place in the whole embryo. In situ hybridization with labeled RNA probes has been used to detect the tissue specific expression pattern of numerous genes. Combination of these tools allows the production of a fairly comprehensive description of the gene expression patterns during zebrafish development. As little is known about spatial and temporal expression of selenoprotein, we found it timely to undertake a systematic analysis of selenoprotein expression during development, taking advantage of the zebrafish system. The zebrafish nucleotide sequences corresponding to the selenoproteins known in other organisms were originally identified by a computer-assisted search (Kryukov et al., 2000). Partial cDNA sequences of twenty selenoproteins were thus obtained and cloned. Digoxigenin-labeled RNA probes complementary to the cDNAs were synthetized and hybridized to zebrafish embryos corresponding to different developmental stages. The hybridization sites were revealed by a chromogenic reaction and the whole embryos were observed.

Several selenoprotein genes (TRxs, Deio1, SEP15) showed a diffuse expression pattern. This reflects a general expression in all cells, in good agreement with their being involved in various cell functions. However, the cellular glutathione peroxidase exhibited a expression pattern restricted to certain tissues, hypothalamus, lateral line ganglia …, suggesting a more specialized function of this gene in the building of these tissues. Several zebrafish selenoproteins are encoded by two genes that evolved by genome duplication. Among those, the two copies of the gene encoding the phospholipid hydroperoxide glutathione peroxidase (PHGPx) or the selenoprotein P were expressed in territories which are mutually exclusive. For example, PHGPx a is expressed in the yolk sack layer, whereas PHGPx b is expressed in the pronephriduct, epiphysis at 48 hours. This result suggests a tissue specialization for each of the PHGPx gene. Regarding selenoproteins of unknown function, expression in certain tissues during development might be indicative of potential functions. For example, we showed that selenoprotein N is expressed in somites during early embryogenesis. This finding can be related to the observation that mutations in this gene cause a form of congenital muscular dystrophy in human (see related abstract in this volume). The selenoprotein X/R is the only transcript that did not show up in the assay.

Kryukov GV, Gladyshev VN. (2000) Genes Cells. 12: 1049–60.

086.—Selenium Regulation and Selenium Function in Second-generation Se-deficient Rats. Roger A. Sunde, Sean M. Blake and Jacqueline K. Evenson. Molecular Mineral Nutrition, Nutritional Sciences and Biochemistry, University of Missouri, Columbia MO.

We are using a second-generation severely Se-deficient (F2) weanling rat model to better understand selenium regulation of selenoprotein expression and the role of selenium in growth. In this model, male weanling pups from Se-deficient (−Se) dams, fed a −Se crystalline amino acid-based diet (3 μg Se/kg diet), grow at half the rate of Se-supplemented (+Se) littermates. To determine how low selenoprotein mRNA levels can fall when growth is impaired, pups from +Se and −Se dams were fed the −Se diet or supplemented with 200 μg Se/kg diet for 28 d. For −Se pups from +Se or −Se dams, −Se pups had liver glutathione peroxidase-1 (GPX1), GPX4 and thioredoxin reductase (TRR) activities were 1.5%, 24% and <1%, respectively, of +Se pups. Messenger RNA levels for GPX1, GPX4, TRR and Sel-P in −Se pups from +Se dams were 10%, 63%, 58% and 59%, respectively, of the +Se pups, and were not significantly further reduced in −Se pups from −Se dams. Because TRR activity but not mRNA drops substantially in −Se rats, we determined the changes in growth and TRR activity in F2 rats in which single small Se injections significantly increase growth. Single litters were fed the −Se diet for 14 d, randomly divided, and then injected intraperitoneally with saline or selenium at 1, 2, or 10 μg Se/100 g BW as selenite. Injection of10 μg Se significantly increased growth 49% and increased liver TRR activity to 20% of +Se levels while GPX1 increased <4% and GPX4 was little affected; muscle TRR activity increased to 32% of +Se levels. These studies show that TRR activity responds to small Se injections more readily than do GPX1 and GPX4 activities.

088.—Functional Consequences of Human Selenium Depletion. Malcolm J. Jackson. Department of Medicine, University of Liverpool, Liverpool L693GA, U.K.

Dietary selenium intake has declined in the UK and other northern European countries for approximately 25 years, but no functional consequences of this decline have been apparent. International epidemiological studies support an increased risk of cardiovascular disease or cancer as a potential consequence of these changes. Previous data indicate that subjects in the UK who took small selenium supplements responded by changes in the activity of some selenium-dependent enzymes and other biochemical measures and our studies have examined whether these changes are accompanied by functional changes in immune status and the handling of a live attenuated RNA virus. Data indicate that provision of small selenium supplements (50 or 100 mg/day) in the form of sodium selenite, induced an increase in measures of cell-mediated immunity that was accompanied by more rapid clearance of the administered modified RNA virus. These studies stress the importance of development of functional measures of nutritional adequacy for assessment of nutritional requirements and support previous suggestions that a diminished selenium supply may play a role in rapid viral evolution.

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089.—Approaches to Improve the Bioavailability of Trace Elements. Lena Davidsson. Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, PO Box 474, CH-8803 Ruschlikon, Switzerland.

The importance of trace element bioavailability in the etiology of nutritional deficiencies can be expected to be most pronounced in individuals with high requirements. Of special concern is the situation in poor communities where infants and young children are consuming monotonous, cereal based complementary foods. Traditionally, cereal based gruels are often one of the first semi-solid foods to be introduced into the infant's diet. These foods can be expected to have low energy and nutrient density as well as low bioavailability of iron and zinc due to the presence of phytic acid. Ascorbic acid is a potent enhancer of iron absorption that can overcome the inhibiting effect of phytic acid when present in high enough quantities. However, home prepared complementary foods based on cereals and legumes contain negligible amounts of ascorbic acid unless ascorbic acid rich fruits are mixed with the cereal or consumed at the same time. Strategies to improve iron and zinc bioavailability from complementary foods need be explored and adapted to local conditions. For example, the impact of enzymatic degradation of phytic acid in cereal based complementary foods and increased consumption of foods rich in ascorbic acid need to be evaluated. In addition, locally available foods with high iron and zinc density should be identified, based on acceptability, affordability etc., and promoted to improve iron and zinc nutrition during early life.

090.—Is Selenium from Animal Sources Bioavailable? Susanne Bügel,* Brittmarie Sandström,* Erik H. Larsen,[+] and Leif H. Skibsted.** *Research Department of Human Nutrition and **Department of Dairy and Food Science, Center for Advanced Food Studies, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark. [+]The Danish Food Administration, Søborg, Denmark.

In areas with a low selenium (Se) content of the soil, Se-intake is often below recommended levels. In order to be able to issue scientifically based recommendations to the public regarding Se-rich food or Se supplements, knowledge about absorption, excretion, storage and effects of the different chemical forms of Se occurring in foods and supplements is needed. The aim of the studies presented was to measure the bioavailability and effect on Se status of Se from animal foods and supplements. Three independent studies were performed. Apparently healthy volunteers, aged 20–30 years participated. Study 1. 12 subjects (3 M & 9F) were fed 100 g shrimps per day for 6 weeks in addition to their habitual diet, resulting in a total intake of 127 ± 5.5 mg Se/d. Study 2. 12 men were fed a controlled diet with pork meat and pork meat products for 3 weeks, total Se intake was 106 ± 13 mg Se/d. Study 3. 27 subjects (7M & 20F) were given 200 mg Se/d as Se-methionin, Se-cystine or Se-yeast to their usual diet for 12 weeks. Total Se was measured in shrimps, diet, feces, urine and plasma by flameless AAS with Zeeman background correction or hydride generation ICP-MS. Glutathione peroxidase (GSH-Px) activity was measured by a kinetic assay using a COBAS Mira. The apparent absorption of Se was 83 ± 4% from shrimps and 94 ± 1% from pork meat. The total retention of Se was 74 mg/d with shrimp intervention and 61 ± 24 mg/d with pig meat intervention. The urinary Se excretion was 16 ± 7% of intake from shrimps (n = 5) and 39 ± 21% of intake from pork meat. Intake of shrimps had a small effect on plasma Se (5%), while no changes were observed when similar amounts of Se were given as pork meat. When Se was given as supplements plasma Se concentration increased in all groups (41–116%). Neither diets nor supplements changed GSH-Px activity in erythrocytes. Despite a high apparent absorption and retention of Se from shrimps and pig meat the availability of Se for blood proteins from these sources appears to be low. In contrast Se from supplements is highly available for blood proteins. This suggests that Se in animal sources is bound in a chemical form that is different from the forms in supplements and might be in an unavailable form.

091.—Marginal Maternal Zinc Intake Affects Mammary Gland and Neonatal Copper Metabolism in Rats. Shannon L. Kelleher and Bo Lönnerdal. Department of Nutrition, University of California Davis, Davis, CA.

Marginal zinc (Zn) intake is more common than previously thought leaving women and infants particularly vulnerable to Zn deficiency and other nutritional consequences. Severe Zn deficiency interferes with copper (Cu) metabolism; however, the mechanisms behind this interaction are unknown. To determine the effects of marginal maternal Zn intake on maternal and neonatal Cu metabolism, we fed rats (n = 6 rats/group) a control diet (25 mg Zn/kg) or a diet marginally low in Zn (10 mg Zn/kg) pre-conception through lactation. Plasma, small intestine, liver, mammary gland and milk Cu and Zn concentrations were measured in dams and suckling pups at d 14. Marginal maternal Zn intake did not alter maternal plasma or tissue Cu or Zn or milk Zn concentrations; however, milk Cu concentration was significantly higher (1.75 vs 2.95 mg Cu/L, P = 0.003). mRNA expression of copper transporter-1 (CTR1),

a Cu import protein, was identified in the mammary gland and dams fed a diet marginal in Zn showed a trend toward higher CTR1 mRNA levels than control rats, while mRNA expression of ATP7A, a Cu export protein, was unchanged. Pups from dams fed the marginal Zn diet had higher small intestine and liver Cu and lower plasma Cu than pups from control dams. These differences may be consequences of an elevated milk Cu concentration, as there were no indications of Zn deficiency in the dams or pups and suggest that mechanisms regulating neonatal Cu transport may not be not fully developed. These results indicate that marginal Zn intake during pregnancy and lactation increases Cu export into milk, possibly through alterations in mammary gland Cu transporter levels. Due to the immature regulation of neonatal Cu metabolism, high milk Cu concentration may potentially expose infants to excess copper.

092.—Iron Deficiency Anemia Reduces Thyroid Peroxidase Activity in Rats. Sonja Y. Hess,* Michael B. Zimmermann,* Myrtha Arnold,[+] Wolfgang Langhans[+] and Richard F. Hurrell.* *Laboratory for Human Nutrition, Institute of Food Science, Swiss Federal Institute of Technology, Zürich, Switzerland and [+]Physiology and Animal Husbandry, Institute of Animal Sciences, Swiss Federal Institute of Technology, Zürich, Switzerland.

Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism. However, the mechanism is not yet clear. The objective of this study was to investigate if iron (Fe) deficiency lowers thyroid peroxidase (TPO) activity. TPO is a heme-containing enzyme catalyzing the two initial steps in thyroid hormone synthesis. Male weanling Sprague-Dawley rats ($n = 84$) were assigned to seven groups. Three groups (ID-3, ID-7, ID-11) were fed an Fe-deficient diet containing 3, 7 and 11_g Fe/g, respectively. Because IDA reduces food intake, three groups were pair-fed to each of the ID groups and one control group consumed food ad libitum, all with Fe-sufficient diets (35_g Fe/g). After 4 weeks, mean hemoglobin, triiodothyronine (T3) and thyroxine (T4) were significantly lower in the Fe-deficient groups than in the control group ($p < 0.001$). By multiple regression, food restriction had a highly significant, independent effect on T4 ($p < 0.0001$), but not on T3. TPO activity (by both guaiacol and iodine assays) was markedly reduced by food restriction ($p < 0.05$). IDA also independently and significantly reduced TPO activity ($p < 0.05$). Compared to ad libitum controls, mean TPO activity per thyroid determined by the guaiacol assay in the ID-3, ID-7, and ID-11 groups was decreased by 56, 45 and 33%, respectively. These data indicate Fe deficiency sharply reduces TPO activity, and suggest that decreased TPO activity contributes to the adverse effects of IDA on thyroid metabolism.

093.—Colonic Cell Copper Content in Young Men Consuming Normal and High Levels of Dietary Copper. Judith R. Turnlund,* Joseph M. Domek,* Padmanabhan P. Nair[+] and Sam J. Bhathena.[#] USDA/ARS, *Western Human Nutrition Research Center, University of California, Davis, CA, USA, [+]Johns Hopkins University, MD, USA, and [#]Beltsville Human Nutrition Research Center, Beltsville, MD, USA.

The role of intestinal cells in copper homeostasis and storage has not been studied. Homeostasis is regulated by absorption, excretion, and storage. The effect of dietary copper on copper absorption, retention, and status has been determined in a number of studies in humans. There is little effect of copper intake on plasma copper, urinary copper, or other indices of copper status. The efficiency of copper absorption is inversely related to copper intake, but more copper is absorbed and retained when copper intake is high. Thus copper retention is partially regulated at the level of absorption. Retention is also regulated via excretion into the gastrointestinal tract. Copper is excreted via the bile and it has been assumed that the increased excretion into the gastrointestinal tract was due to biliary copper. It seemed possible that some of the absorbed copper is sequestered in the intestinal cells. To test this hypothesis we measured the copper content in colonic cells isolated from stool samples of 9 young men when their dietary copper was 1.7 mg/d and after 4.5 months of copper supplementation, when intake was 7.8 mg/d. The mean copper content of the cells, expressed as mg copper per gram of cell protein, was 0.78 vs 1.65 mg/g (SEM 0.19) when the normal and high copper diets, respectively, were consumed ($p > 0.05$). In contrast to the copper content of plasma and urine, copper in intestinal cells increased significantly when intake was high. It is likely that copper was sequestered by these cells following absorption and did not get into systemic circulation. Thus, the amount of endogenous copper in stools is probably a combination of biliary copper excretion and copper sequestered in sloughed intestinal mucosa. This suggests that retention of copper by the intestinal cells increases when dietary copper is high and may play a role in copper homeostasis.

094.—An Adaptational Response of Protein Calorie Malnutrition on Zinc Transporter in Intestinal Brush Border Membrane of Rhesus Monkeys: a Kinetic Study. R. Prasad and R. Nath. Dept. of Biochemistry Postgraduate Institute of Medical Education and Research, Chandigarh–160012, INDIA.

Introduction: Zinc plays a fundamental role in the expression of genetic potential, synthesis, repair and structural integrity of genetic material via acting an essential component of vide variety of metalloenzymes, transcription factor like zinc finger proteins and other proteins (1). Zinc deficiency is prevalent among Indian malnourish children. Therefore, the present study was conducted to investigate the zinc metabolism in rhesus monkey, phyllogenetically close to humans. Method: Protein calorie malnutrition was induced in rhesus monkey as described previously (2). Brush border membrane was isolated from proximal part of Intestine and Zinc transport. Study were conducted as described before (2, 3). Results: Zinc levels in different organs like Intestine, Kidney and liver of PCM monkeys were significantly lower than controls. Notwithstanding steady state Zn2+ uptake in intestine by tissue accumulation method as well as intestinal brush border membrane vesicles (BBMV) from PCM monkeys were significantly higher than controls. Further, kinetic analysis revealed that the increased uptake into BBMV is associated with increase maximal velocity (Vmax) of zinc transport system. Discussion: Decreased levels of zinc in different organs from PCM monkeys could be associated due to restriction of diet in PCM monkeys. Strikingly, increased uptake of Zn2+ into BBMV from PCM monkeys is initricated with increased Vmax, reflecting either increase in the total

number of Zn2+ transporters or increase the number of active Zn2+ transporter: In conclusion, the present study suggest that induction of increased Zn2+ uptake capacity in intestinal BBMV from PCM monkeys is the compensation mechanism for maintaining zinc homeostasis.

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095.—Identification and Functional Characterization of Selenium-containing Proteins using Bioinformatics Methods. Vadim N. Gladyshev. *Department of Biochemistry, University of Nebraska, Lincoln, NE.

Recent completion of human and other genomes provided the first view of genome organization and identified the majority of genes in these organisms. A variety of bioinformatics methods can be employed to identify additional genes and generate functional predictions for a variety of proteins. Selenium-containing proteins are encoded by genes containing in-frame UGA codons for selenocysteine. Since UGA is recognized as termination signal by currently available annotation programs, the majority of selenoprotein genes present in sequence databases are incorrectly annotated. We developed computational methods that allow identification of selenoprotein genes by searching for sequence, structural and thermodynamic parameters of mRNA structures located in untranslated regions of selenoprotein genes. Using these methods, the majority of selenoprotein genes in eukaryotes and archaea was identified. Subsequent computational analyses of new sequences for gene occurrence profiles, phylogenetic patterns, gene expression profiles and patterns of domain fusion generated functional predictions. A specific example of such analyses will be discussed: 1) Selenoprotein R was initially identified as a selenoprotein with no homology to functionally characterized proteins; 2) Functional predictions implicated this protein in the pathway of methionine sulfoxide reduction; 3) Experimental analyses of these predictions revealed a biological function of Selenoprotein R.

096.—Identifying New Genes Involved in Copper Homeostasis Using a Drosophila Genomics Approach. J. Camakaris, M. Norgate* and P. Batterham. Department of Genetics University of Melbourne, Victoria, 3010, Australia.

The vinegar fly, Drosophila melanogaster, has been the model system in which many of the universal principles of eukaryotic genetics have been elucidated. The Drosophila genome has been sequenced and is well annotated. 78% of human genes have a D. melanogaster homolog. We are using three complementary approaches that should permit novel genes involved in copper homeostasis to be identified and cloned, in studies recently commenced. The first of these uses chemical mutagenesis to randomly generate point mutations throughout the genome. The subsequent screening of mutants on lethal concentrations of copper will permit resistant mutants to be identified. Mutagenesis so far has resulted in isolation of 11 resistant strains from a screen of 650,000 embryos on 4 mM Cu and mapping is in progress. A second approach employs a library of 196 strains each heterozygous for a different but overlapping large chromosomal deletion mutation and provides the potential to screen ~80% of the genome for loss of function effects that lead to copper sensitivity or copper resistance. Screening to date of 73 deletion strains has revealed ten resistant and one sensitive strain. In the case of one of the strains we have used overlapping deletions to narrow the region containing the resistance gene to approximately 6 Æ 35 genes. The third approach we are using is gene chip technology to analyze the pattern of expression of all genes in the Drosophila genome for organisms grown under different conditions of exposure to copper. The various approaches being used are complementary and should all lead to gene identification and isolation.

097.—Design, Implementation and Meta-analysis of Field-based Studies in Developing Countries (DCs). Sunil Sazawal,* Janet M. Peerson and Rosalind S. Gibson.#** *All Indian Institute of Medical Sciences, New Delhi, India, **University of California, Davis, USA, #University of Otago, Dunedin, New Zealand.

This workshop will consider the design, implementation, and meta analysis of field-based trace element studies in DCs using zinc as an example. Both dietary assessment and randomized double-blind controlled trials (RCT), used to define the public health importance of zinc deficiency and formulate strategies for its prevention, will be reviewed. RCTs are a powerful research tool, when designed and used correctly, for establishing causal relationships between deficiencies of trace elements such as zinc and adverse functional health outcomes. The randomization eliminates bias arising from baseline confounding variables, and the double blinding avoids bias due to unintended interventions and ascertainment bias. Six steps in the design of RCTs: (i) sample selection; (ii) measurement of baseline variables; (iii) randomizing the participants; (iv) applying the intervention; (v) following up the cohort; (vi) measuring the outcomes, together with strategies to ensure their implementation, will be reviewed. Approaches for ensuring community support, randomization, quality control, blinding, as well as monitoring compliance and drop-outs throughout the RCT will be highlighted. Analysis via intention-to-treat, subgroup analysis, and early stopping rules will be considered. Results from these individual RCT's can be combined through meta-analysis or pooled analysis in an effort to gain a better understanding of the population relationship. The steps required for conducting a meta-analysis (identification, selection, abstraction and analysis) will also be reviewed, using as an example, summary statistics from the effects of 37 individual zinc supplementation RCT's on linear and ponderal growth. The application of such analyses has enabled the positive impact of zinc supplementation on height as well as reduction in the rates of diarrhea and pneumonia to be confirmed.

098.—Integrating Trace Element Metabolism from the Cell to the Whole Organism. Dennis J. Thiele. University of Michigan Medical School, Ann Arbor, Michigan.

The redox chemistry of copper (Cu) makes this both a powerful enzyme catalyst and a dangerous reactant which generates hydroxyl radical. While virtually all cells from microbes to mammals must acquire Cu to drive important biochemical

reactions, the potential toxicity of Cu demands an exquisite level of vectorial transport and homeostatic control. Our laboratory is interested in how organisms acquire Cu through the action of high affinity plasma membrane Cu transporters of the Ctr class of proteins. We have isolated Ctr Cu transporters from baker's yeast and fission yeast, from flies mice and mammals. This presentation will focus on understanding how the Ctr high affinity Cu transport proteins function from their biochemical mechanism of action in yeast and cultured metazoan cells, to their roles in Cu delivery and mammalian embryonic development.

099.—Trace Element Biology: the Knowledge Base and its Application for the Nutrition of Individuals and Populations. Vernon R. Young. Laboratory of Human Nutrition, School of Science, MIT, Cambridge, MA 02139, USA.

Impressive strides are being made in the understanding of trace element metabolism and function accelerated, in part, by the sequencing of genes and of whole genomes and by application of the reductionist approach for exploring the molecular nature and mechanistically defined response of gene-trace element pathways to changes in the trace element profile of cells. We know, to make a point and by way of examples, (i) that zinc stabilized domains mediate protein-protein, protein-nucleic acid and protein-lipid and that there are as many as 706 human genes that encode C2H2 zinc fingers, (ii) that zinc serves as a cofactor in all six classes of enzyme, (iii) that the copper uptake molecule, Ctrl, plays a crucial role in mammalian Cu homeostasis and (iv) the uptake of iron, that plays a critical role in the immune system and defense against infection, occurs by modulating transferring receptor mRNA stability through the binding of Fe-regulatory proteins to the 3′-untranslated region of specific genes. This is impressive. However, and not so impressive, (i) the precise recognition of mild trace element deficiencies and how to establish their functional consequences, possibly confounded by concurrent trace-element inadequacies, are difficult to assess, (ii) approaches to the quantitative determination of requirements for trace elements remain unsatisfactory and archaic, in so many ways, (iii) our understanding of the biological basis for the variation in requirements among apparently similar individuals is poor and (iv) determination of the qualitative and quantitative extents to which genetic, epigenetic and dietary factors interact to determine the nutritional phenotype, all remain exciting challenges for those interested in trace element/nutrition research. I will provide some ideas as to how we might embrace, in the context of a reconstructive approach, the exciting new knowledge and related techniques emerging during the post-genome era to develop new paradigms for assessing trace element needs, status and for establishing effective nutrient intake under different conditions of complex genotype-environment interactions. Metabolites are functional cellular entities and I will argue for a vigorous application of metabolomics and of metabolic profiling that is linked with genomics, proteomics and trace element kinetics and systems control analysis as components of the new paradigm. An interdisciplinary approach also will be necessary and this raises the issue of the training of the next generation of TEMA scientists and the nature of the required research support, which I will also touch upon. Finally, this presentation is intended to be complimentary to those given by other plenary speakers, where our common goal is to promote, through expanded biological knowledge and its effective application, the enhanced role of trace elements for human well-being.

105A.—In Vitro Penetration and Permeation Studies of Multitracer in Everted Small Intestinal Sacs of Control and Streptozotocin-diabetic Rats. W. J. Ding, S. Enomoto, R. Hirunuma, T. Ohyama and K. Igarashi. Multitracer Group, Cyclotron Center, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351–0198, Japan.

Altered tissue concentrations of trace elements have been reported in streptozotocin (STZ)-induced diabetic rats. To determine whether increased and/or decreased levels of metal elements were associated with intestinal transport and absorption was studied in control and STZ rats using the multitracer techniques and an improved everted duodenum-jejunum sac system. A radioactive multitracer solution was prepared from a silver target irradiated with a 14N beam of 135 Mev/nucleon accelerated by the RIKEN Ring Cyclotron. In this study, the multitracer solution was dissolved into a tissue culture medium (Medium 199, Sigma, USA). Diabetes was induced in male Sprague-Dawley rats at 8 weeks of age (260–280 g) with a single intravenous dose of streptozotocin (55 mg/kg body weight).The body weight, water consumption and food consumption, and blood glucose levels were routinely monitored once a day for 1 wk. Rats were killed with a sodium pentobarbital overdose (250 μg/kg), the duodenal and jejunal segments were everted and divided into 3 cm length sacs. Each sac was filled with Medium 199, and then immersed in 20 ml of Medium 199 containing an appropriate amount of the multitracer solution and incubated with bubbling 95% O2 and 5% CO2 at 37°C for 5, 15, 30, 60, and 120 min, respectively. In this study, the intestinal hyperplasia in STZ rats was observed, and significantly affected the intestinal function. It is likely that diabetes directly affects Na+-K+ATPase activity on the intestinal membrane. Furthermore, trace elements absorption and transport in duodenal and jejunal segments were significantly affected by the altered intestinal function in diabetic rats. Decreased element transport is due to increased accumulation within the enterocyte. Briefly, these results suggest that the membrane physical composition of the intestinal epithelium is an important regulatory site for absorption and transport of elements.

106A.—Antioxidant Capacity and Vitamin C Concentrations in Dogs. Chris Charlton, Sally Carlin, David Martin, Tina Callcut and John Rawlings. WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, LE14 4RT, U.K.

Oxidative damage resulting partly through free radical insult has been associated with many age-related diseases. The body has a number of mechanisms to resist oxidative damage, including antioxidant enzymes and macromolecules such as ceruloplasmin and ferritin. This study investigates the antioxidant capacity in dogs using the ferric reducing antioxidant power method1

(FRAP) to determine total plasma antioxidant capacity, and the ferric reducing antioxidant power and ascorbic acid concentration assay (FRASC) as an indirect measure of plasma vitamin C concentration. Plasma samples (0.5 ml) were obtained from 55 healthy dogs of 12 different breeds to establish normal ranges. Acceptable intra and inter coefficients of variation (CV < 10%) were obtained for both methods. Mean FRAP and FRASC results and normal ranges are shown below:-

	FRAP (mmol/l)	FRASC (mmol/l)
Mean	394.75 ± 92.97	39.08 ± 12.04
Normal range (mean ± 2SD)	208.81–580.69	15.00–63.16

Results suggest a difference in total antioxidant status between breeds. The established normal ranges may be used to assess canine antioxidant capacity. However further research is recommended to determine if breed specific normal ranges are required.

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107A.—Arsenic Species in Eggs from Laying Hens Fed with a Diet Containing As_2O_3. Vekoslava Stibilj, Ingrid Falnoga and Darija Gibičar. Department of Environmental Sciences, "Jožef Stefan" Institute, Ljubljana, Slovenia.

Arsenicals are sometimes used as feed additives for poultry to promote growth and as chemotherapeutics, and in agriculture as insecticides, pesticides and herbicides. In order to study arsenic species, we performed experiments with laying hens exposed to As2O3, which is one of the most toxic forms of arsenic often used as a pesticide. 18 Rhode Island Red hens, aged 49 wk at the time of caging, were divided into three groups of six, the control and two test groups. The exposed groups were fed with As enriched feed (7.5 and 30.0 mg/g) for 19 d. The addition of arsenic to feed did not effect feed consumption, the body weight of the hen, nor the egg production in the trial (1). No changes were observed in essential element tissue concentrations compared to control group (2). Eggs were collected on the 12th, 13th, 14th and 17th, 18th, 19th days of the experiment and prepared as composite samples of yolk and white separately from the 12th to 14th days and from the 17th to19th, six yolk and six white samples from the three groups. Total As concentration was determined by RNAA and arsenic species were determined by a modified method described by Sŏlejkovec (3). In all cases of white samples from the test groups, regardless of the number of extractions, the ratio of methanol/ water mixture and temperature of extraction, about 70% of total As was extracted and 84.6% As was present as dimethylarsinic acid (DMAA). In yolk, a preliminary extraction with diethyl ether was made before the methanol- water extraction due to high fat content. About 95% of total As was extracted in all cases and 85.0% was present in the form of DMAA. In water-methanol extracts from yolk and white samples, no other anionic or cationic arsenic compounds were detected. We can conclude that methylation to DMAA was the main detoxification mechanism for arsenic, and that the dose did not exceed the methylation capacity.

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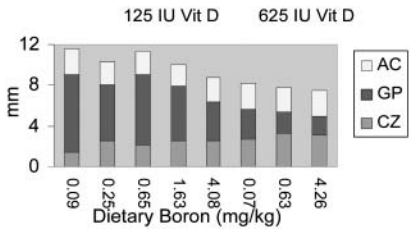
108A.—Estimation of Dietary Boron and Silicon Intake in China. Junquan Gao. Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing 100050, China.

This paper describes dietary intake of Chinese boron and silicon. Dietary boron (B) intakes were estimated from Chinese Total Diet Study (TDS) in 1992~1993. The second Chinese total diet study was carried out in the same survey areas and method of the first total diet study(1). Dietary boron intake of other six countries selected the availability of adequate food consumption survey dada and nutrient databases. The comparison of adult males average daily boron intake was estimated by linking the database with the survey food records as well as TDS. Mean dietary intake estimates for adult males in the United States, Germany, Great Britain, Mexico, Kenya, Egypt, and China were 1.11 ± 0.69, 1.72 ± 0.91, 1.30 ± 0.63, 2.12 ± 0.69, 1.95 ± 0.57, 1.31 ± 0.50 and 1.37 mg B/d respectively. The major contributors to dietary boron intake were identified in each country. The top contributors in the United States, Germany, Great Britain, Mexico, Kenya, Egypt, and China were coffee (6.5%), wine (14%), wine (15.4%), tortillas (56.1%), maize (35.3%), rural breads (27.4%) and cereals (36%) respectively. These dietary boron intake estimates provide data that will be useful for setting recommended daily intake levels when boron is confirmed to be essential in humans. The average daily dietary silicon intake of the Chinese adult males was estimated by Chinese TDS. Mean dietary silicon intake was 43.15 mg Si/d. The major contributors to dietary silicon intake were cereals (55.5%), vegetables (23.3%), legumes (5.9%), and potatoes (5.8%) in China. These dietary silicon intake estimates provide data that will be useful for setting recommended daily intake levels when silicon is confirmed to be essential in humans.

109A.—Dietary Boron Alleviates Growth Cartilage Abnormalities Induced by Vitamin D Deficiency in Chicks. Curtiss D. Hunt. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202 USA.

Previous research has shown that dietary boron affects several physiological indices in vitamin D (Vit D) deficient chicks including growth cartilage maturation. Furthermore, boron, independent of Vit, D, had an effect on the morphology of the mineralization zone of the chick growth plate. The objective of this experiment was to characterize the interaction between boron and Vit D as it affects bone histology. Day-old cockerel chicks (20 per group) were fed a ground corn-casein-corn oil basal diet containing 0.08 mg B/kg and adequate in all other vitamins and minerals. Five of the dietary treatments were 125 IU Vit D3/kg (inadequate) and supplemental boron (as H3BO3) to achieve final concentrations of 0.09, 0.25, 0.65, 1.63, or 4.08 mg B/kg, all within a physiological range. The other three dietary treatments were 625 IU Vit D3/kg (adequate) and

supplemental boron to achieve 0.07, 0.63, and 4.26 mg B/kg. The chicks were exposed to 24 hr fluorescent ceiling light with an intensity of 200 lux. At age 28 days, the dissected right proximal tibiae were sectioned in the coronal plane through the center of the medial and lateral tubercles and fixed with 10% neutral buffered formalin and stained with Alcian blue. Midpoint heights of the articular cartilage (AC), and of the epiphysial growth plate (GP) and calcified zone (CZ) were obtained by image analysis at low level magnification (~6.5 X). The data were analyzed statistically by linear regression analysis based on the natural log of the dietary boron concentrations. A range of dietary boron affects growth plate morphology in Vit D-inadequate, but not Vit D adequate chicks. For example, pro-



gressive increases in the amount of dietary boron added to a low-boron diet provided overall reduction in the heights of the abnormally thickened growth plate (GP); ($P < 0.001$; $R2 = 0.16$). Concurrently, the dietary treatments improved height of the calcified zone (CZ); ($P < 0.03$; $R2 = 0.07$). In other words, dietary boron ameliorated the defects in cartilage maturation and calcification induced by Vit D deficiency. These findings provide further indirect evidence that dietary boron enhances the metabolic utilization of Vit D. Because dietary boron apparently provides beneficial effects to the vitamin D deficient chick, further research is necessary to establish the range of dietary boron that maximizes growth plate maturation.

110A.—Investigation of Borate Addition to NAD+/NADH Using Electrospray Ionization Mass Spectrometry (ESI-MS) and 11B-NMR. Danny H. Kim,* Beth N. Marbois,* Kym F. Faull[+] and Curtis D. Eckhert.* *UCLA Department of Environmental Health Sciences, Los Angeles, CA and [+]UCLA Psychiatry and Biobehavioral Sciences, and The Neuropsychiatric Institute, Los Angeles, CA.

The current understanding of borate (B(OH)4-) interactions with cellular targets is based on indirect spectrophotometric analysis performed under non-physiological conditions. For example, it is known that high concentrations of borate inhibit coenzyme dependent enzyme catalysis such as dehydrogenase activity. In this paper we characterized nucleotide and borate esters using electrospray ionization-mass spectrometry (ESI-MS) and 11B-NMR spectroscopy. Our study objectives were to examine pH dependent esterification of boric acid and borate with NAD+ and NADH: (1) directly by ESI-MS and (2) directly by 11B-NMR spectroscopy at physiological concentration. The analysis demonstrated that both borate mono- and diesters formed with NAD+. Borate diol monoester and diester shifts were observed at d7.80 and d12.48, respectively with 11B NMR. ESI-MS showed that a 1:1 addition of borate to NAD+ was preferred over a 2:1 addition. The boric acid/borate ester peak areas were pH dependent, but borate esters predominated even below the pKa. The cis-diol on the ribose ring was the active binding site, not the phosphate backbone. Fragmentations of the complexes were evaluated using tandem mass spectrometry (MS/MS) to determine if borate preferred a specific ribose ring in 1:1 addition. From the MS/MS data, it was determined that boric acid/borate bound to the ribose ring adjacent to the adenine group. In conclusion, these data show that esterification of borate with NAD+ under different conditions can result in different three-dimensional coenzyme structures. (Supported by a gift from US Borax).

111A.—Dietary Boron Alters the Effect of Different Amounts of Dietary Omega-3 Fatty Acids on Growth and Bone Physical Characteristics of F1 Generation Rats. Forrest H. Nielsen. U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND.

Female and male Sprague-Dawley rats weighing between 160 and 180 g, and 190 and 210 g, respectively, were fed diets containing about 70 μg boron (B)/kg in a factorial arrangement with supplemental B at 0 (deficient) and 3 (adequate) mg/kg and canola oil (CO) or palm oil (PO) at 75 g/kg of diet as variables. After five weeks, six females per treatment were placed with males to breed. Dams and pups continued on their respective dietary treatments through gestation, lactation, and after weaning. Twelve F1 rats per treatment for each sex were designated for bone breaking determinations. At 13 weeks after weaning, when dietary fat was PO, the weights of boron-deficient rats were less than boron-adequate rats. Feeding CO instead of PO decreased the weights of B-adequate females; with males, weights were decreased in B-adequate, but increased in B-deficient animals. An interaction between dietary fat source and B affected several parameters of bone breaking. For example, stress (force per unit area of the cross section of bone at breaking) was decreased in B-deficient rats when CO was fed instead of PO with the effect more marked in females. Stress was increased in B-adequate rats when CO was fed instead of PO. The interaction between dietary B and fat source affected several physical characteristics at the point the bone was broken. For example, when CO was fed instead of PO the lateral width was increased in B-deficient rats, but decreased in B-adequate rats. Bone mineral composition was not affected by an interaction between dietary fat source and B. Boron deficiency markedly reduced the B concentration and resulted in small decreases in calcium, phosphorus, zinc and potassium concentrations, and feeding CO instead of PO resulted in a small decreases in iron and magnesium in bone. The results support the hypothesis that B and omega-3 fatty acids affect similar systems in higher animals including physical characteristics of bone.

112A.—U.S. Food Consumption Patterns show Diets with Fruits, Nuts and Legumes are High in Boron. Charlene J. Rainey and Leslie A. Nyquist. Food Research, Inc., Costa Mesa, CA, USA.

Our boron intake estimates from NHANES III and CSFII 1994–96 for the dietary intake assessments were reported by

the Food and Nutrition Board. Results were slightly higher than our previous estimates of dietary boron intakes. NHANES III median adult boron intakes range from 0.75 to 1.35. However, a previous study of six countries revealed that boron intakes for adult males were lower in the U.S. (using CSFII 1989–91) than in Great Britain, Germany, Mexico, Kenya, and Egypt. Using the boron results from adult male participants in NHANES III, high boron intake was associated with a risk reduction of prostate cancer. The adult male mean daily boron intakes within quartiles 1, 2, 3, and 4 were 0.497, 0.923, 1.361, and 2.629 mg, respectively. In the 4th quartile, fruits/fruit juices contributed 27.8% of total boron, beverages 24.0%, vegetables 12.5%, grains 10.8%, and nuts 9.4%. When comparing adult males in the 1st quartile of boron intake with those in the 4th quartile, the food consumption patterns showed the largest differences in increased nut, fruit, and legume consumption. Mean fruit consumption increased in each quartile, from 28 g in the 1st quartile to 327 g in the 4th quartile. The mean nut consumption increased from 1g in the 1st quartile to 19 g in the 4th quartile. Fruits and nuts have especially high boron concentrations. Increased fruit and nut consumptions have been associated with the risk reduction of cancer, heart disease and prevention of bone decay. The health benefits of diets rich in fruits and nuts are commonly attributed to the vitamin C, vitamin E, potassium, and magnesium from these foods. Yet dietary guidance to increase fruit and nut consumption would result in substantially increased daily boron intakes. Further research is needed to determine boron's role in a healthy diet.

114A.—Prospective Study of Hair Calcium Concentration as a Predictor of Mortality from Coronary Heart Disease. Jozsef Bacso* and Allan MacPherson.[+] *Institute of Nuclear Research, P.O. Box 51, H-4001, Debrecen, Hungary and [+]SAC, Auchincruive, Ayr, KA6 5HW, Scotland, UK.

As reported at TEMA 10 hair calcium concentration was shown to be inversely related to CHD and to reliably reflect the known pattern of heart disease risk in the UK. Its usefulness as an individual risk factor has yet to be demonstrated. An opportunity to do this arose when hair samples were obtained from 822 male subjects aged from 25–64 years who had been recruited by the Belfast Centre for the WHO co-ordinated MONICA project (multinational monitoring of trends and determinants in cardiovascular disease). These samples which were collected between November 1983 and May 1984 were analyzed by us in 1994. Data on the number of deaths and the causes of mortality over the period from 1983−2000 were collected and collated during 2001. Over this seventeen-year period 112 subjects died. Almost 75% of the deaths were due either to some form of malignant neoplasm or of cardiovascular disease. Subjects who died of acute myocardial infarction had significantly lower hair calcium concentration (459 ± 2.81 ppm) than the mean concentration of all other subjects (582 ± 0.81 ppm). However the mean concentration of subjects who died of the various forms of malignant neoplasms (428 ± 2.61 ppm) was even lower and also significantly below that of the survivors. Thus low hair calcium concentration would appear to suggest increased risk of death but not to afford a differential prognosis as to the likely cause. A full analysis will be presented showing the effect of the inclusion of other risk factors on these findings.

115A.—Increases in Dietary Calcium Intake in Urban Children at High Risk of Lead Poisoning. John D. Bogden,*[+] Francis W. Kemp,* Carol Penn-Erskine,* Venus Coba,* Peter Wenger,* Debra Palmer-Keenan,[+] Marijane R. Lundt, Amy Davidow* and Donald B. Louria.*** *Department of Preventive Medicine and Community Health, UMDNJ-New Jersey Medical School, Newark, NJ, USA, [+]Department of Nutritional Sciences, Rutgers University, New Brunswick, NJ, USA, and **Gateway Maternal and Child Health Consortium, Newark, NJ, USA.

Increased dietary calcium (Ca) reduces lead (Pb) absorption and toxicity in experimental animals and adults, and may also do so in children. We surveyed dietary Ca intakes of 314 Newark, NJ, children in 1997–98 and 540 children between 6/1/00 and 3/22/01 during the first stage of a campaign to increase diet Ca intakes of the target population. Most were African-American or Hispanic. For 1–3 year old children, mean (± SE) Ca intakes increased from 746 ± 34 (n = 175) in 1997–98 to 869 ± 27 (n = 319) mg/day in 2000–01 ($p = 0.0057$). For 4–8 year old children, mean intakes increased from 739 ± 36 (n = 139) in 1997–98 to 866 ± 36 (n = 221) mg/day in 2000–01 ($p = 0.018$). The proportions with intakes below the DRI were similar in 1997–98 and 2000–01 for 1–3 year olds (31.4 vs. 32.5%) and 4–8 year olds (59.0 vs. 58.8%) children. Higher than expected percentages of 1–3 and 4–8 year old children had Ca intakes below 300 mg/day for both time periods (10.3–15.0%). Lower ($p = 0.020$) Ca intakes were found in children whose parent's reported lactose intolerance (671 ± 81 mg/day, n = 33) than if they did not (885 ± 23 mg/day, n = 479). These data suggest that low budget community efforts may increase mean Ca intakes, but decreasing the percent of urban children with low intakes is a greater challenge, especially if there is concern about lactose intolerance. Actual or perceived lactose intolerance may contribute to the high prevalence of pediatric lead poisoning in urban areas. (Supported in part by the Healthcare Foundation of New Jersey, Dairy Council Middle Atlantic, and the Foundation of UMDNJ.)

117A.—Calcium Absorption from Bread Fortified with a Milk Mineral Isolate in Pre- and Post Menopausal Women. Lisbeth Grinder-Pedersen,* Mikael Jensen,[+] Esben Skipper Sørensen, Brittmarie Sandström,* Liselotte Højgaard[++] and Marianne Hansen.*** *Research Department of Human Nutrition/ LMC Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark. [+]Department of Mathematics and Physics, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark, **Protein Chemistry Laboratory, Department of Molecular and Structural Biology, University of Aarhus, Science Park Aarhus, Denmark. [++]Dept Clin Physiol Nuclear Med, The National University Hospital, Copenhagen, Denmark.

Ca bioavailability from milk is generally high, which may be explained by the presence of bioactive substances such as casein peptides and lactose. Consequently, a preparation of Ca and other milk constituents isolated from milk may show similar high Ca bioavailability and thus be an alternative to CaCO3 for fortification. The aim of the study was to compare Ca absorption from bread fortified with a Ca-containing milk mineral isolate (MM) to the fortification with CaCO3 in pre- and

postmenopausal women. Furthermore, Ca absorption from single meals was compared to 1-day diets in postmenopausal women. All data are presented as mean ± SD. Ca absorption was measured in 9 premenopausal (23.7 ± 2.8 y) and 20 postmenopausal (64.1 ± 4.8 y) women. Single meals based on bread fortified with A) MM 1, B) MM 2 and C) CaCO3 (control), 1-day diets comprising of two single bread meals fortified with D) MM 1 and E) CaCO3 (control) were extrinsically labeled with 47Ca and absorption estimated from the measurement of whole-body retention and urinary 47Ca excretion. The bread was fortified with 160 mg Ca. The premenopausal received A, B and C (n = 9) while the postmenopausal received A, C and D (n = 10) or A, C and E (n = 10) in a randomized cross-over design. In the premenopausal women, Ca absorption from single meals was significantly lower from MM 1 (23.4 ± 3.8%) (P = 0.02) and marginally lower from MM 2 (24.6 ± 4.4%) (P = 0.05) compared to CaCO3 (29.9 ± 4.6%). The same was found in postmenopausal women, MM 1 (21.6 ± 6.0%) vs. CaCO3 (28.7 ± 7.4%) (P < 0.02). Absorption of Ca from MM 1 in single meals did not differ between the pre- and postmenopausal women (P = 0.47). In the postmenopausal women, Ca absorption from 1-day diets (22.9 ± 3.2%) did not differ from single meals (21.6 ± 6.0%) (P = 0.7) after fortification with MM 1 or fortification with CaCO3: 24.2 ± 6.5% (single meals) vs. 28.7 ± 7.4% (1-day diets) P = 0.18. In conclusion, fortification of bread with a MM did not show a higher Ca absorption in pre- or postmenopausal women compared to CaCO3. The use of single test meals in measuring Ca absorption appears to reflect the absorption from a total diet. Supported by the Danish Dairy Research Foundation and The Danish Research Development Program for Food Technology.

118A.—Dietary Inclusion of Amaranth (Amaranthus Gangeticuss) Greatly Reduces Calcium but not Zinc Turnover from Bangladeshi Diets. T. Larsen,* S.K. Biswas,[+] I. Tetens and S.H. Thilsted.**** *Dept. of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Denmark. [+]Grain Quality and Nutrition Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh. **Research Dept. of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark.

Bangladeshi national surveys reveal that the daily food intake mainly consists of polished rice supplemented with small amounts of vegetables, fish, pulses and meat. This composition can be improved nutritionally, and greater amounts of minerals and vitamins can be supplied by increasing the non-rice fraction of the diet. The present study investigated the effect of supplementating rice with different levels of the green leafy vegetable, lal shak (Amaranthus gangeticuss), fish and lentils. Balance experiments were conducted using young, growing Wistar rats during a 28 days' period. Furthermore, deposition of calcium and zinc in various organs and tissues obtained after killing the animals was studied, as well as analysis of indicative blood parameters was carried out. Calcium intake varied among groups (10 mg/d for the rice diet vs. 49 mg/d for a recommended rice-lal shak-fish-lentils diet). However, Ca absorption was inhibited in diets containing the vegetable, lal shak (fractional absorption 0.40 vs. 0.93 in a similar diet without lal shak, p < 0.001). On the contrary, absorption of zinc was not affected by the inclusion of Amaranthus; the fractional absorption was 0.28 vs. 0.30. Bone parameters such as mass, mass in proportion to body weight, fractional mineralisation, and elemental calcium and zinc content, were all significantly lower in diets containing Amaranthus, on average 6–13% lower. Soft tissue content of zinc was not reduced by the inclusion of Amaranthus; liver, muscle and kidney tissue contained the same level of zinc. Supplementation of rice with lal shak, fish and lentils generally led to an increased intake of minerals and trace elements as well as an increased absorption. However, attention must be paid not only to the gross mineral content but also to inhibitors of mineral bioavailability in the vegetables locally available and commonly used in the Bangladeshi diet. The inhibitor of calcium but not zinc absorption in Amaranthus has yet to be identified, but oxalic acid may be a potential candidate. The difference between the affinity of calcium and zinc to the inhibitor and thus the formation of complexes that take place in the intestinal lumen may explain the present results.

122A.—Implementation of the Salt Iodisation Program in Ethiopia: Problems and Prospects. Yodit Abebe, Jeya Henry and Conor Reilly. Oxford Brookes University, Headington, Oxford OX3 OBP, UK.

IDD is a problem of considerable public health significance in Ethiopia (1). Though salt iodisation was successfully implemented in the late 1980s, due to various factors, including war, today iodisation has ceased. The situation is presently improving but much remains to be done. To assist the improvement a survey has been undertaken to review the problem, with special reference to salt iodisation. The aim was to obtain information on factors that currently affect the IDD program. A questionnaire was distributed to a variety of government authorities, including the National Micronutrient Committee (NMC), on operation of the program. Additional information was obtained in focus group discussions to assess public knowledge, attitudes and practices relating to IDD and use of iodised salt. A market survey investigated availability and cost of the salt. Responses indicate that though steps are being taken to implement the program, progress is slow (2). It is recognized that iodisation of domestic salt is the most appropriate method for controlling IDD, but this requires more dedication from all sectors. Focus group discussions indicate a lack of awareness regarding IDD and its prevention. Iodised salt is available only in supermarkets in cities and is much more expensive than non-iodised salt. Results demonstrate an urgent need for a national plan of action for the introduction of iodised salt as well as for better information and education at all levels about the IDD problem and its consequences.

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2. Ministry of Health (1999) Micronutrient Deficiencies Control Programme in Ethiopia – Profile Draft. Ministry of Health, Addis Abeba, Ethiopia.

123A.—Effect of Nitrate (NO3-), Nitrite (NO2-), and Allylisothiocyanate (SCN-) on Thyroid Functions in Rats and Alleviating Properties of Iodine. Renata B. Kostogrys,* Pawel M. Pisulewski,* Anna Pecio[+] and Elżbieta Sikora.* *The Agricultural University, Faculty of Food Science and Technology, Department of Human Nutrition, Krakow,

Poland, and [+]The Jagiellonian University, The Faculty of Biology and Earth Sciences, Department of Comparative Anatomy, Krakow, Poland.

A two-factorial (4x2) design was used to study the effect of four goirogen treatment (control, nitrite, nitrate, and allylisothiocyanate), and two iodine levels (2 μg I/rat/day; 4 μg I/rat/day) on the growth performance, thyroid hormones (fT4, TSH) and thyroid morphology. Forty-eight five-week old rats, weighing on average 95 g, were divided equally into eight dietary groups. The animals were fed the AIN (1993) diet, that provided either 2 μg/d or 4 μg/d iodine (KIO3), as follow: control, control+NaNO2 (25 mg/100 g), control+NaNO3 (300 mg/100 g), and control+allylisothiocyanate (6 mg/100 g). The body mass of rats was monitored weekly. On the d 18, the rats were anaesthetised with ether and their blood was drawn by cardiac puncture. The immulite rat TSH application kit was used to determine TSH concentrations in blood serum. Serum fT4 was determined according to the LIA method. The thyroid glands were processed by the conventional paraffin technique. The mean height of 100 follicle epithelial cells was measured. Data were tested by two-way ANOVA. The rat growth was not affected by the goitrogens. Serum fT4 concentration was reduced by all goitrogens in rats with 2 μg iodine (I control-24,6; II-21,3; III-21,8; IV-19,8 pmol/l respectively). In contrast, serum TSH level was significantly increased after administration of NaNO2 ($P < 0,05$), and SCN- ($P < 0,02$). The histological examination of the thyroid gland showed a series of morphological alterations (tall follicular epithelial cells and reduced amount of colloid). On the other hand, in the rats receiving the same doses of goitrogens, but supplemented with iodine (4 μg/rat/d), all parameters became very similar to the control group. The results indicate that dietary nitrate, nitrite and allylisothiocyanate impairs thyroid metabolism in rats and leads to thyroid hypertrophy. At the same time the goitrogenic effects of these ions can be alleviated by iodine supplement.

124A.—A New Strategy to Fight Iodine Deficiency Disorders. Walter A. Rambeck, Dietmar Ranz, Mao L. He, Mathias von Lukowicz, Reinhard Reiter, Rüdiger Arnold, Herve le Deit, Robert Aquaron and Marc David. Institute for Animal Physiology, Physiological Chemistry and Animal Nutrition, Ludwig-Maximilians-University Munich, Veterinaerstr. 13, D-80539 München, Germany.

Iodine deficiency disorders are still a problem in some parts of Europe and additional strategies to fight iodine deficiency disorders are necessary. These approaches include the addition of iodine or iodine containing algae to animal feed, thus improving the iodine content of milk, meat and eggs. Absolutely new is the idea of supplying fresh water fish with iodine rich algae, which in addition contain also other essential trace elements, vitamins and omega-3 fatty acids. Algae supplemented fish feed was given to trouts and chars in feeding trials which lasted between 3 and 12 months. Brown algae (Laminaria digitata) containing up to 4 g iodine per kg of dry matter were added to the fish feed in a concentration of up to 0.8%. Every four weeks 20 fishes were slaughtered and analyzed for their iodine content. In addition, we analyzed the quality parameters of the fish meat, i.e. firmness, color, and pH-level. Iodine was measured by the Sandell-Kolthoff method as well as by a gaschromatographic method and by neutron activation analyses. Already after 8 weeks the iodine in fish fillet increased from 140 μg/kg fish in control fish to 568 μg. There was no influence on the quality parameters of the fish. Taste of the fish, as tested by a panel of 12 persons, did not differ at all between control and algae fed fish. In order to find out if the carry over of iodine from plant to fish to man is functioning, 14 volunteers received either 200 gram control fish or algae fed fish. The iodine excretion via urine which is a good parameter for iodine intake increased significantly from 69 μg to 101 μg iodine/g creatinine when algae fed fish was eaten. In summary, the results indicate that the proposed strategy is well capable to increase iodine intake in man and thus to fight iodine deficiency disorders.

125A.—Molybdenum Balance Studies in Infancy. Erika Sievers, Urte Schleyerbach* and Jürgen Schaub. *Christian-Albrechts University Department of Pediatrics, Kiel, Germany.

Low Mo concentrations in human milk and a considerable range of the molybdenum (Mo) concentration in infant formulas have been described (Biol Neonate 1991;59:201–203). However, data about the metabolism of this essential trace element in infancy are still limited. Therefore, Mo balance studies were conducted in term infants fed an infant milk formula with a concentration of 50 μg Mo/L (unsupplemented). Five healthy term ($n = 22$ balances) and three preterm infants (gestational age 36 weeks, $n = 12$ balances) were investigated at the ages of 15, 35, 57 85 and 113 (± 3 days), respectively. Collections comprised a period of 72 hours. Results refer to the median values of all investigation periods of each child. Specimens were analyzed by atomic absorption spectroscopy. A daily Mo intake of 8.9 (7.8–10.5) μg Mo/kg body weight resulted in an urinary excretion of 4.3 (4.0–5.6) μg Mo/kg and a fecal excretion of 0.67 (0.53–0.99) μg Mo/kg. The Mo retention was 3.6 (2.8–5.3) μg Mo/kg. The percental urinary Mo excretion was 51.9 (38.8–71.1) %, the percental fecal excretion 8.5 (7.2–12.1) % and the percental the apparent retention 40.5 (31.5–49.9) % of the intake. These results confirm that Mo is well absorbed from infant formulas and render no support for additional supplementation. Mo concentrations exceeding those of human milk by far should be avoided.

126A.—Magnesium in Erythrocytes, a Sensitive Marker of Individual Status as shown by Drinking Mineral Water. Maurice J. Arnaud,* Jean-Marc Millot,[+] Stéphane Sebille,[+] N. Beljebbar,[+] M. Peirera,[+] Magalie Sabatier,* J. Caron[#] and Michel Manfait.[+] *Perrier Vittel Water Institute, Vittel, [+]Lab. Spectro. Biomol., UFR Pharm., Reims, [#]Med. Int., CHR Maison-Blanche, France.

There are no sensitive and convenient clinical tests to assess magnesium (Mg) status. After a previous study showing in migraine patients that lower intracellular blood cells Mg values were improved in patients with the lowest levels when drinking a Mg-rich mineral water, a similar study has been performed on 24 healthy volunteers (11 women and 13 men aged 40 years, range 28–59). Total Mg in plasma (TP), erythrocytes (TE) and lymphocytes (TL) and Mg2+ in plasma (FP) and lymphocytes (FL) were analyzed before and after 2

and 4 days drinking 1L/day of a Mg-rich natural mineral water (Hépar®,110 mg/l Mg). Measurements were performed by atomic absorption spectrophotometry, confocal UV-micro-spectro-fluorometry using the fluorescence emission of the Mag-Indo-1 probe and by potentiometry with a ion-selective electrode. After 4 days, significant increases in total plasma Mg (+5%, $P < 0.01$), total lymphocyte Mg (+15%, $P < 0.02$) and total erythrocyte Mg (+6%, $P < 0.001$) were observed while Mg2+ plasma concentration was unchanged. The influence of the initial Mg value on the Mg increase showed for only for TE Mg that the highest increases corresponded to the lowest initial Mg values, whereas no significant variation occurred for the highest initial TE Mg. This suggests that the Mg supplementation induces Mg increases in erythrocytes up to the maximum concentration of 2.2 mM. This study demonstrates the significant short-term increases in Mg concentrations in healthy volunteers after the oral absorption of Mg-rich mineral water. Erythrocyte Mg concentration was shown to be the most sensitive parameter modified in relation to individual Mg status.

127A.—The Effect of Magnesium Creatine Chelate on Ergogenic Performance. Ashmead, H.D., Bourdonnais, A. and Ashmead, S.D. Albion Advanced Nutrition, Clearfield Utah USA, Albion Advanced Nutrition, Clearfield Utah USA, Albion Advanced Nutrition, St. Brieuc, France.

Supplements of either magnesium or creatine have previously been reported to improve ergogenic performance. The purpose of this study was to compare ergogenic activity in groups of rats after receiving no supplement or supplements of creatine monohydrate alone, creatine monohydrate plus MgO, creatine monohydrate plus magnesium bis-glycine chelate, or a chelate of magnesium creatine. The magnesium creatine chelate was a 1:1 (Mg:creatine) chelate. Except for the control group, daily dose was 5 mg magnesium and 100 mg creatine monohydrate/kg body weight/day for 8 days. Following 8 days of supplementation weights (5% of body weight) were attached to each rat and it was swam to exhaustion. Following a 30 minute recovery period, each rat was swam to exhaustion a second time. The source of the supplement appeared to affect ergogenic performance as seen below. The magnesium creatine chelate not only resulted in significantly ($p < 0.01$) greater swimming time to exhaustion in the first period, but it was the only supplement that produced significant ($p < 0.05$) ergogenic recovery during the second swimming period. It was concluded that when magnesium is chelated to creatine monohydrate, the resulting molecule may provide greater ergogenic activity than when the two are supplied as admixtures.

Group	Swim 1 (sec.)	_A (sec) (%)	Swim 2 (sec.)	_B (sec) (%)	%B – %A
1 Control	178.8 ± 10.1		158.0 ± 5.9		
2 Creatine	233.7* ± 16.2	54.8(30.6)	234.02* ± 7.1	78.5 (49.7)	19.1
3 Creatine+MgO	204.0 ± 14.4	25.2(14.1)	196.0 ± 24.4	38.0 (24.0)	9.9
4 Creatine+MgAAC	229.3* ± 20.1	50.5(28.2)	229.4* ± 27.9	71.4 (45.2)	17.0
5 Mg Creatine Chelate	243.6**± 13.4	64.8(36.2)	249.0* ± 9.8	91.0 (57.6)	21.4*

* $p < 0.05$
** $p < 0.01$

128A.—A Kinetic Model of Magnesium Metabolism in Healthy Men Based on Two Stable Isotope Tracers. Magalie Sabatier,* Frederic Pont,# William R. Keyes,+ Maurice Arnaud* and Judith R. Turnlund.+ *Water Institute Perrier Vittel, Vittel, France, and #Mass Spectrometry Unit, IFR 30, Toulouse, France, and +Western Human Nutrition Research Center, USDA/ARS, University of California, Davis, CA, USA.

There are few kinetic models of magnesium (Mg), however this mathematical description of mineral distribution can provide useful information and may potentially be used as biomarkers of whole body mineral status. The aim of this work was to build a compartmental model of Mg to describe Mg pools and their characteristics, the absorption rate and fecal endogenous excretion. The study was carried out in 6 healthy adult men. After 6 days of adjustment to a 3-day rotating diet, subjects received an oral dose of 70 mg of 26 Mg in water with breakfast and 30 mg of 25 Mg was infused. Blood, urine and feces were collected for 12 days. Isotopic ratios were determined by Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Data were analyzed using a compartmental model proposed by Avioli and Berman (1) for each subject using the SAAM II (Simulation Analysis and Modeling, Seattle, WA) program. This analysis permits the exploration of 24% of total body Mg that exchanges rapidly. The model consists of one plasma compartment and 3 extravascular pools. The plasma compartment represents less than 1% of total body Mg. One of the extravascular compartments contains 79% of exchangable Mg that turns over in 115 hours. The two others turn over in 0.4 and 9 hours. Fractional Mg absorption (0.44 ± 0.07; mean ± SD) was not significantly different from the results obtained by fecal monitoring. The average (± SD) endogenous fecal excretion was 47 ± 11 mg per day. Those results are in agreement with the literature. The absence of difference between methods for Mg absorption determination demonstrates the reliability of the approach.

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129A.—Mg in a Group of Postmenopausal Women: Influence on Some Cardiovascular Risk Factors. Veiga L.,* Ferreira A.,+ Moreira H., Monteiro C.P.,+ Gonçalves A.,++ Sardinha L.,++ Bicho M.$^{\phi}$ and Laires M.J.+** *ESTeSL, Lisbon; +Biochemistry Lab., FMH, UTL, Lisbon; **Sports Dep., UTAD, Vila Real; ++Exercice and Health Lab., FMH, UTL, Lisbon; $^{\phi}$Genetic Lab., FML, UL, Lisbon, Portugal.

Cardiovascular pathologies (CP) have often been associated with magnesium (Mg) deficiency or depletion1. This study aims

to determine the association between Mg levels, insulin resistance, lipid profile and body composition parameters in a group of 61 Caucasian sedentary postmenopausal women, aged between 50 and 77 years. Diabetics, hypertensives and women on hormonal replacement therapy were excluded. A three days food record was used to collect nutritional information. Red blood cell's (RBC-Mg) and plasma Mg (P-Mg) were determined by atomic absorption; fasting insulin by RIA; fasting glucose, triglycerides (TG), total, HDL and LDL cholesterol (C) by spectrophotometry; body mass index (BMI) was derived from weigh and height; body fat percent; abdominal fat and fat free mass were assessed by Dual-energy X-ray absorptiometry. The group was divided according to RBC-Mg $<$ 44 mg/l (G1, n = 40) and RBC-Mg $\geq$ 44mg/l (G2, n = 21)2. According to RDA, Mg intake was normal. Concerning body composition parameters no significant differences were found between the two groups, but it should be noted that in average, women in this study were obese (BMI = 27.7 $\pm$ 3.9 Kg/m2)3. Independent sample student's t-test showed significantly higher insulin (p = 0.036); P-Mg (0.004); RBC-Mg (p = 0.000) and TG (p = 0.014) in G2. Although not statistically significant, glucose; HOMA; total-C and LDL-C were higher and HDL-C was lower in G2. Considering our results we may suggest that both groups have a high risk for development of CP. G1, since they have extremely low RBC-Mg; G2, although presenting higher RBC-Mg, it is still significantly lower than normal reported values (p = 0.000)4. Associated with this, G2 has significantly higher TG, which may be related to remnant lipoproteins, which in turn can contribute to increase the risk for the development of CP. G2 also presents a trend to higher total-C and LDL-C and lower HDL-C, which are the major determinants of coronary heart disease. Considering that Mg intake is normal, these results suggest that menopause is associated with metabolic deregulations that might lead to a low Mg status. This, especially when associated to obesity, leads to a high risk of development of insulin resistance and eventually CP.

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4. Laires, M.J. & Cunha Monteiro, A.L. (1980) Rev. Port. Bioq. Aplicada 3, 145–153.

130A.—Manganese Content of Plant-based Beverages in Comparison to Infant Formulae. Kevin A. Cockell, Giuseppe Bonacci and Bartholomeus Belonje. Nutrition Research Division, Food Directorate, Health Canada, Ottawa, Canada.

While breastfeeding is best for infants, there are circumstances where infant formula is used as an alternative. In some instances homemade infant formulas based on milk or milk "substitutes" may be used, though this practice is discouraged by the FDA and by Health Canada. Plant-based beverages (PBB) represent one type of product that might be fed to infants, though they are not nutritionally complete for infant feeding. Several (but not all) PBB products bear label statements that the product is not a substitute for infant formula. Soybeans accumulate manganese (Mn) from the soil in which they are grown and soy protein contains relatively high levels of Mn. We have compared the Mn content of 36 soy-based beverages (SBB), 5 rice-based beverages (RBB), 6 evaporated milks, and 30 infant formulas (14 soy-based and 16 milk-based products), all obtained from local commercial sources. Triplicate samples were dry-ashed and analyzed for Mn by flame atomic absorption spectrometry. SBB contained the highest levels of Mn (16.5 $\pm$ 8.6 μg/g dry wt, mean $\pm$ sd), followed by RBB (9.9 $\pm$ 1.7 μg/g dry wt). Individual PBB products had Mn concentrations ranging from 2–16 times the mean Mn concentration of the soy-based infant formulae (2.4 $\pm$ 0.7 μg/g dry wt) and 7–56 times the mean Mn concentration of the milk-based infant formulae (0.70 $\pm$ 0.35 μg/g dry wt). Evaporated milk contained very little Mn (0.02 $\pm$ 0.03 μg/g dry wt). Calculated mean Mn intakes from PBB by infants up to six months of age, assuming complete substitution of these products for infant formula (0.78 L/day), approached the Tolerable Upper Intake Level (UL) for 1–3 year olds (no UL has been derived for Mn in infants under 1 year of age). Calculated as μg Mn/100 kcal (based on label energy content of each product), the PBB contained mean Mn concentrations 5–7 times the levels found in the soy-based infant formulae, and exceeded the range derived from UL values and typical energy intakes for 1–3 year olds. Children 1–3 years old do not typically rely on a single food such as PBB for their nutrition. Since they contain comparatively high concentrations of Mn, and are in any event nutritionally inadequate as sole sources for infant feeding, PBB products like these should not be used as substitutes for infant formula.

131A.—Effect of High Manganese Intake and Iron Deficiency in Infant Rats on DMT-1 Expression and Tissue Mineral Accumulation. Trinh T. Tran, Shannon L. Kelleher and Bo L. Lönnerdal. UCD, Department of Nutrition, Davis, CA.

The Mn level in infant formula is much higher than in human milk, thus infants are exposed to variable levels of Mn when Mn homeostasis is incompletely developed. High Mn can be toxic and may be exacerbated by anemia, which is prevalent during infancy. DMT-1 transports Fe and Mn and is regulated by Fe status. We studied effects of Mn intake in control and anemic rat pups on tissue Mn and Fe, and DMT-1 expression. Pups were supplemented with 0, 250, or 500 g Mn/d from birth through d14. Tissue Mn concentration (small intestine, liver and brain) was positively correlated with Mn intake and was exacerbated by anemia. Tissue Fe concentration was negatively correlated to Mn intake in anemic but not control rats, indicating a significant interaction between Mn intake and Fe status on tissue Fe concentration. Although Mn supplementation decreased DMT-1 mRNA expression in the small intestine of anemic rats (−60%) it was increased in control rats (200%). Thus, excess Mn intake during infancy causes tissue Mn accumulation which is more pronounced with anemia. These effects occur despite decreased DMT-1 expression suggesting additional mechanisms for Mn absorption that may be dependent upon Fe status.

132A.—An In-vitro Bioavailabilty Study on Selenium: Effect of Selenium Supplementation on Total Trace Element and Species Levels in Human Body Fluids. Jill Adair,* Neil. I. Ward,* Fadi R. Abou-Shakra[+] and Heather Walker.[+] *ICP-MS Facility, Department of Chemistry, University of

Surrey, Guildford, Surrey, UK and +Micromass UK Ltd., Floats Road, Wythenshawe, Manchester, UK.

Selenium is a key component in a number of functional selenoproteins, required for normal health, e.g. antioxidant glutathione peroxidase. Selenium has a high biological activity and its bioavailability and toxicity is heavily dependent on the chemical form available. An in-vitro bioavailability study, which mimics the human gut, was therefore performed on certain commercial selenium supplements (in triplicate) in order to determine both the total amount and the chemical form of selenium, which is available for absorption from each of these supplements. A pilot study was also carried out alongside the bioavailability study. This involved a subject group of 4, consuming these supplements. The levels and species of selenium within the blood serum, urine and seminal plasma of the subject groups then investigated over the period of the trial, namely one month. The analysis was performed using high performance liquid chromatography (HPLC) coupled with collision and reaction cell inductively coupled plasma mass spectrometry (ICP-MS). Data will be presented in relation to the bioavailability of each of the commercial selenium supplements and on the effect that consumption of these supplements has on the total and species levels of selenium within the human body fluids.

133A.—Effect of Stress on Trace Element Levels in Human Blood Serum in Relation to the Success or Failure of IVF Treatment. Jill Adair,* Neil. I. Ward* and Andrew Horne.+ *ICP-MS Facility, Department of Chemistry, University of Surrey, Guildford, Surrey, UK and +IVF Unit, London Hammersmith Hospital, Du Cane Road, London, UK.

Female infertility can occur because of several biological and environmental factors, e.g. genetic, nutritional, exposure to toxic chemicals, social drugs (alcohol and cigarette smoking) and stress (hormonal balance and fertile period). Lappé (1983) stated that at least 19 elements, namely Li, B, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, In, Te, I, Hg and Pb could cause potential problems in human fertility, embryogenesis and pregnancy. Several pilot studies in our laboratory have shown that trace elements may also play a vital role in fertilisation (IVF treatment. As it has long been known that stress can cause imbalances in the levels of certain trace elements, analysis of Fe, Cu, Zn, Se, Cd and Pb in particular could provide a link between the levels of stress and the success or failure of IVF treatment. Blood serum was sampled from fifty female infertile IVF patients and analyzed for both hormone (cortisol, beta-endorphin, catecholamine and prolactin) and trace element content). Sample preparation involved microwave digestion (using a 1:10 serum: 70% Aristar™HNO3 ratio and analysis was by PSN-ICP-MS after dilution with distilled deionised water (25 cm3). The elements 57Fe+, 65Cu+, 66Zn+, 82Se+, 112Cd+ and 208Pb+ were investigated and method validation utilized Seronorm™ Trace Element Serum reference material. Trace element data will be presented in relation to various stress factors.

1. Lappé in "Trace Elements in Health". Rose, J. Ed, Butterworth, p231.

135A.—Are Target Trace Element Requirements Being Met in Home Intravenous Nutrition (HIVN)?–a Two Centre Survey. M. Baines,* J. Shaffer,+ D. Barber, A. Forbes,++ S. Gabe** and A. Shenkin.*** *Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool L7 8WP, UK, +Hope Hospital, Salford and St Mark's Hospital, London.**

Guidelines for trace element provision to HIVN patients are evolving. This survey studied how 2 UK centers (A and B) were meeting current recommendations.

Patients (23 and 22 respectively) receiving at least 50% of their nutritional requirement via IVN were recruited. Blood samples were taken at entry and repeated 12–74 weeks later, dependent upon recall. Plasma samples were analyzed for Cu, Se and Zn by atomic absorption spectroscopy. Results are tabulated below:

Element	Ref. range (plasma) (mmol/L)	Current IV Recom'd μmol/week	Mean intake/week μmol (range)		Final plasma concn mean (sd) μmol/L		P (1 v 2)
			Centre A	Centre B	Centre A1	Centre B2	
Copper	12.1–24.7	35–140	122.7 (0–140)	128.0 (80–140)	17.2 (3.37)	17.3 (2.73)	P = 0.903
Selenium	0.7–1.6	2.8–5.6	2.6 (0.4–2.9)	5.1 (2.9–5.9)	0.81 (0.27)	1.06 (0.33)	P = 0.03
Zinc	11.0–20.0	350–700	609 (0–700)	751 (420–840)	14.7 (4.09)	15.9 (5.17)	P = 0.475

6 patients from Centre A had plasma Se concentrations <0.7 μmol/L despite having mean Se intakes not significantly different from the remainder of group. Cu and Zn appear to be adequately provided at both centers, whereas Se provision differed and was possibly inadequate for some patients at Centre A. Both the absolute amount and the frequency of supplementation may need to be considered in such patients.

136A.—Element Concentrations in Lenses and Aqueous Humour: an Experimental Cataract Study in Rabbit. Laura Ciaralli,* R. Giordano, M. Ciprotti, A. Sepe, P. Rossi, F. Cruciani,° A. Moramarco° and S. Costantini. Istituto Superiore di Sanità–Applied Toxicology Dept.–Trace Elements Unit, Viale Regina Elena, 299-00161 Rome, Italy, °II Div. Oculistica, Institute of Ophtalmology, University "La Sapienza", Viale del Policlinico, 00161 Rome, Italy.

The determination of inorganic ions in cataractous human lenses has been the subject of several investigations but data are

sometimes still contradictory. Recent epidemiological studies performed by the Ophthalmologic Institute of Rome have evidenced that the senile cataract represents the main cause of hospitalisation for surgical reason. This should be searched for a general progressive ageing of the population and the improvement of surgical techniques. Thus, the capacity in preventing the lenticular opacity or simply in delaying the cataract development should represent a great success both from human and economic point of view. The aim of the present work was to evaluate whether an animal model of induced cataract could cause changes in the mineral content of lenses. This would represent a good way to study the behavior of the elements in cataractous lens, overcoming at the same time ethical aspects and the difficulty of obtaining normal human lenses to be used as controls. Some elements, namely Ca, Na, K, Cu and Zn, were determined in lenses of eight experimental rabbits; the cataract was induced by a Laser treatment of the right eye of the animals. In consideration that the aqueous humor surrounding the lens is the source of nutrients necessary for lens metabolism, and that it constitutes a pathway for the elimination of metabolic products, we have also determined the concentrations of the above ions in the aqueous humor. The methods used for the analytical determinations of the elements were both flame and flameless atomic absorption spectrometry. The changes of the element concentrations calculated in lenses of treated eyes showed significant increases of Ca (8,9x, $p < 0.001$), Cu (1.9x, $p < 0.004$) and Na (3.1x, $p < 0.01$) than controls. Changes not significant were instead observed for zinc and potassium, and only a tendency to decrease was evidenced. The concentration of the elements in the aqueous humor resulted significantly lower in treated eyes of the rabbits than in controls (Ca 20.0 mg/l vs 32.4 mg/l, $p < 0.001$; Na 0.70 g/l vs 1.84 g/l, $p < 0.001$; Cu 0.042 mg/l vs 0.074. $p < 0.002$; Zn 0.08 vs 0.12: $p < 0.001$) with the exception of potassium which gave a decrease statistically not significant. The greatest decrease was observed for Na (−61.9%), whereas the other elements gave lower percentages (Ca-38.2%; Cu-42.8%; Zn- 33.3%). The results indicate that this model is suitable for studying some aspects of cataractogenesis.

137A.—Heavy Metals in Ayurvedic Preparations: Analytical Problems and Toxicological Aspects. M. Ciprotti, R. Giordano, L. Ciaralli, A. Sepe, P. Rossi and S. Costantini. Istituto Superiore di Sanità – Applied Toxicology Dept. – Trace Elements Unit, Viale Regina Elena, 299 Rome, Italy.

Ayurveda is a traditional form of Indian medicine that is practised mostly in many countries in the Asian Pacific Region. In recent years, the system has spread to the west, due to the increasing diffusion of oriental meditative practices and the interest toward their cultural and philosophical background. In addition to the use of herbs having medicinal properties, Ayurveda uses minerals and metals. In many European countries these products generally do not require medical prescriptions. Toxicological problems rise by the fact that the consumer often considers these products to be "natural" and therefore rarely associates their use with possible collateral effects. Thus, some cases of intoxication referable to the presence of heavy metals in ayurvedic preparations also were observed in Italy. The aim of this work was to determine some elements, namely arsenic, cadmium, chromium, iron, mercury and lead in a series of herbal products found on the Italian market. Seventy samples were analyzed. Some analytical problems occurred because of the complex nature of the samples containing both vegetal and mineral matrices. A digestion method using a high-pressure microwave system was applied in order to completely dissolve the samples; the determinations of As, Cd, Cr and Pb were performed by grafite furnace atomic absorption spectrometry, whereas the iron was determined using the flame method. The mercury was analyzed by means of a specific hydride generation apparatus. The acquired data demonstrated that the concentrations of the examined metals were generally within the limits of safety; nevertheless, some samples collected from 1994 to 1996 showed very high values particularly for mercury (8400 mg/Kg) and lead (145000 mg/Kg). Probably, during the above period the Indian manufactures were still not sensitized to the necessity to comply with the regulations of the Western countries. Moreover, other samples had metal concentrations almost reaching the safety limits indicated by International Organism. Therefore, they may become toxicologically important if the accumulation capacity of heavy metals is considered. The work evidenced that during the last few years the quality of the ayurvedic preparations imported in Italy has undergone an improvement, probably due to the action undertaken toward the Italian importers. However, the presence of metals not intentionally added but naturally present in the herbs, or caused by a casual contamination which may occur during the productive cycles, is still a significant aspect that needs to be taken into consideration.

139A.—Hair as a Biopsy Material in Assessing Nutriture and Intoxication. Leslie M. Klevay, Dale M. Christopherson and Terry R. Shuler. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND, USA.

Introduction: Trace elements in hair are being measured and advice on treatment–either supplementation or detoxification–is being given. It is possible to measure trace elements in hair to satisfy the skeptical chemist, but analytical utility in diagnosis, prognosis and therapy generally has not been validated by the usual method of clinical research (1). Method: Hair was collected with some regularity from the occiptonuchal region of a healthy, middle-aged man between February 1968 and December 1986 to evaluate within-person variability of data on several elements. Two shampoos low in copper and zinc were used exclusively; neither prescription medicines for long periods nor nutritional supplements were taken. There was no unusual occupational exposure to elements. After washing with ether, sodium lauryl sulfate detergent in demineralized water and acetone, samples were dessicated and dissolved in nitric acid and hydrogen peroxide for spectroscopic analysis.

Data for six elements are in the table: g/g~,. S.D., (coefficient of variation, %).

Essential nutrients		Potential intoxicants	
Copper	14..610.8 (74)	Aluminum	3.5.14.26 (121)
Selenium	0.53.50.177 (33)	Cadmium	0.16.90.118(69)
Zinc	16.528 (17)	Lead	1.4.90.80 (53)

n = 64, except for aluminum, 63; selenium, 60; and lead, 55.

The coefficients of variation generally are several fold larger than those for similar analysis on a standard hair sample from China. Aluminum, cadmium, copper and lead decreased in the interval; selenium increased. No seasonal effects were detected. Medical, nutritional or toxicologic statements based on analysis of a single sample are of dubious validity because of within-person variability and possible age effects.

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140A.—Effect of Low Doses of Supplementary Iron on Iron, Calcium, Magnesium, Zinc and Copper Status in Iron-Deficient Rats. Krejpcio,* Smol,[+] Twardowski[+] and Wójciak.* *Department of Human Nutrition and Hygiene, August Cieszkowski Agricultural University, Poznan, Poland, [+]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland.

The aim of this study was to evaluate the influence of supplementation with Fe compounds on Fe, Ca, Mg, Zn and Cu status in Fe-deficient rats. The study was carried out on 50 growing male Wistar rats in 2 experiments. In experiment 1, rats were made Fe-deficient by feeding ad libitum Fe-depleted diet (5.8 mgFe/kg d.m.) for 8 weeks, while the controls received Fe-adequate diet (48.9 mgFe/kg d.m.). In experiment 2, Fe-deficient rats were subdivided into 4 groups (equal mean Fe-hemoglobin level in each group) and were given the same Fe-depleted diet supplemented with or without equal doses of Fe (0.3 mgFe/rat/day, equivalent of 30% of daily Fe recommendation for rats) in the form of ferrous sulfate, ferrous lactate, or plant ferritin preparation for 10 consecutive days. At the end of experiment rats were sacrificed, blood was collected by heart puncture, liver, kidneys, spleen and heart were harvested and fixed-frozen for micronutrient (Fe, Ca, Mg, Zn and Cu) analyses. The content of metals in serum and tissue samples was determined by flame AAS method after wet digestion in ultrapure nitric + perchloric acids. For statistical evaluation of results ANOVA and Tukey's tests at $P < 0.05$ were applied with the help of Statgraphics software. Results: It was found that Fe-deficiency led to significant depletion of Fe stores and alteration of Ca, Mg, Zn and Cu concentrations in some organs and tissues of rats. Supplementation with small doses of Fe markedly increased Hb concentration in blood but did not affect serum, liver and kidney Fe, however increased splenic Fe and decreased heart Fe in Fe-deficient animals. Bioavailability of Fe from plant ferritin preparation enriched up to 40% in pure ferritin turned out to be comparable with ferrous sulfate, but higher then ferrous lactate. Supplementary Fe did not affect Ca and Mg status, whereas increased serum and liver Zn in Fe-deficient animals. Supplementary ferritin preparation significantly increased serum, kidney and splenic Cu in Fe-depleted rats. We conclude that Fe depletion leads to impairment of Ca, Mg, Zn and Cu status. Supplementation even with small doses of iron can improve impaired balance of these minerals.

141A.—Trace Elements in Marine Mammal Tissues Archived in the US National Biomonitoring Specimen Bank. Elizabeth A. Mackey, Rabia D. Oflaz, Barbara J. Porter, Stephen A. Wise, and Paul R. Becker. National Institute of Standards and Technology (NIST), Gaithersburg MD and Charleston SC, USA.

The National Biomonitoring Specimen Bank (NBSB) at NIST contains cryogenically preserved environmental specimens that were collected in a clean and well-documented manner, which represent that status of the environment at the time of collection. The inventory of the NBSB includes human liver tissue, human diet, human serum, bivalves, fish, sediment, and marine mammal tissues. Beginning in 1987, tissues were collected from Alaskan marine mammals through a collaboration with the National Oceanic and Atmospheric Administration and the US Department of the Interior and beginning in 1991 tissues were collected from marine mammals from the coasts of the contiguous 48 states also in collaboration with NOAA. To date, tissue have been collected from 33 species, 660 individual animals. Blubber, liver, and kidney tissues are collected and morphometric data and any physiological information are recorded. To date, sub samples of liver and kidney tissues from 93 animals have been analyzed to determine levels of ≤30 trace elements. Trace element data were analyzed to determine whether there are any differences among the species, or geographic regions. Findings include: 1.) levels of silver are orders of magnitude higher in beluga whale (Delphinapterus leucas) liver than in other species; 2.) in many species, selenium, silver, and mercury accumulated in liver with age; 3.) cadmium accumulated in two marine mammal species; 4.) levels of vanadium were much higher in all species of Alaskan mammals than in the three Atlantic east coast mammal species studied; and 5.) the highest levels of calcium were associated with kidney fibrosis in rough-toothed dolphins (Steno bredanensis).

142A.—Low-dose Iron Supplements in Pregnancy Prevent Iron Deficiency at the End of Pregnancy and During the Postpartum Period: the Results of a Randomised Controlled Trial. Maria Makrides,* Caroline Crowther,[+] Robert Gibson,* Murray Skeaff[#] and Rosalind Gibson.[#] *Child Health Research Institute and Adelaide University Dept Paediatrics, Women's & Children's Hospital, Adelaide, Australia; [+]Adelaide University Dept Obstetrics & Gynaecology, Women's & Children's Hospital, Adelaide, Australia and [#]Department of Human Nutrition, University of Otago, Otago, New Zealand.

Background: Controversy surrounds the need for iron supplementation during pregnancy in developed countries with some advising routine supplementation while others screen for anemia and treat if detected. Our aim was to assess the effect of iron supplemented at 20mg/d, a level designed to meet the recommended intakes during pregnancy. Maternal iron status, gastrointestinal side effects and maternal wellbeing were the primary outcomes. Methods: Randomized, double blind, controlled trial comparing a 20mg/d iron supplement with an identical placebo from 20 wk gestation until birth. Findings: At the end of pregnancy, fewer women from the iron group had IDA than the placebo group (6/198, 3% vs 20/185, 11%; RR 0.28, 95% CI, 0.12, 0.68, $p < 0.005$) and fewer women in the iron group had ID than placebo treated women (65/186, 35% vs 102/176, 58%; RR, 0.60, 95% CI, 0.48, 0.76, $p < 0.001$). There were no differences between the groups in the numbers of women reporting nausea, stomach pain, heartburn, vomiting,

rash, hard stool or frequency of bowel actions. SF-36 scores for each of the 8 health concepts assessed did not differ between groups at any time. At 6 months post-partum fewer women from the iron group had ID compared with the placebo group (31/190, 16% vs 51/177, 29%; RR 0.57, 95% CI 0.38, 0.84, $p < 0.005$). The rate of IDA between the groups did not differ. Interpretation: Supplementing the diet of women with 20mg/d of iron from 20 wk of pregnancy may be an important low risk strategy to prevent IDA and ID.

143A.—Nutritional Evaluation of Brazilian Water Polo Athletes: a Multivariate Analysis of Data. Eliane T.L. Mari,* Fátima A.A. Sardinha,* Ana P.S. Soares,* João J. Leite, Jorge E.S. Sarkis[+] and Célia Colli.*** *Fac. of Pharmacy; **Heart Institute, Fac. of Medicine, Univ. of S.Paulo, [+]IPEN/CNEN, S.Paulo, Brazil.

In order to evaluate if physical activity influences mineral nutriture a group of 13 water polo athletes and non athletes women of 17–31 years of age were selected. Hemoglobin (Hb), serum ferritin (FER), %Transferrin Saturation (%ST), VO2max, VO2AT, % AT/ Max and serum concentration of Ca, Mg, Fe, Cu and Zn were determined. A matrix of 312 results was created and a multivariate Cluster Analysis (Ward) and an inferencial analysis were performed in order to identify differences between the groups. The dendogram showed two separated groups: one with 85% of the athletes (G1) and the other with 85% of the control group (G2). The variables that influenced this separation were %ST, FER, VO2 max, VO2 AT and %AT/ Max. The analysis of the other variables such as age, weight, height, BMI, %fat, Energy Intake, Energy Expenditure and macro and micronutrients intake showed that only the Average Energy Expenditure was different (G1 > G2) between the groups ($p < 0.05$). 27%(3/11), 64%(7/11) and 18%(2/11) of the control (G2) showed correspondly %ST, FER e Hb below the normal range, 45% (5/11) of them had Fe deficiency and 18% (2/11), iron deficiency anemia. The group of athletes were adequate in terms of iron nutriture. The indicators of physical activity were efficient in the diferenciation of trained athletes from the controls and the Fe parameters as well. Although the % fat and the mineral concentration in serum didn't influence the separation of both groups there were signs ($p > 0,15$) that with a bigger sample the serum Fe, the serum Ca and the %fat could also be determinants of the separation of the two groups. This kind of analysis can be useful in the identification of subtle diferences that cannot be detected with the univariate analysis.

Acknowledgments: PRONUT; CAPES, CNPQ; FAP; Mario Sérgio Rossi Vieira

146A.—The Multi Element Profile of Human Hair in Croatia–a Cross Sectional Study. Berislav Momčilović* and Anatoly V. Skalny.[+] *Institute for Medical Research and Occupational Health, P.O.B. 291, Zagreb, CROATIA, and [+]Center for Biotic Medicine, P.O.B. 96, Moscow, Russia.

The usefulness of trace element (TE) hair analysis for the assessment of human nutritional status and environmental exposure is controversial. Here we report the results of an analysis of 23 TE in a cross-sectional study of 199 men and 730 women (totaling 929) between 7 and 50 years of age, from the 6 largest Croatian cities. They were concerned about their health status and thought that TE hair analysis could help to reveal a potential problem. Hair samples were washed, digested and analyzed for TE using the inductively coupled argon plasma atomic emission spectroscope (Onischenko et al, Modern equipment and methods of analysis in sanitary and hygienic investigation, FGUP Intersen, Moscow, Russia, 1999). The analytical results for all TEs (*essential) were categorized as Normal (N), Excessive (E), or Deficit (D). The sum of E+D represents the Imbalance. The frequency distribution of 21367 analyses is as follows: N = 16664 (78.0%), D = 2368 (11.1%), E = 2335 (10.9%), I = 4703 (22.0%).

Result\Element		*Mn	*Na	*Mg	*K	*Fe
*Se	*Zn	*Cr	Si	*Cu	*Ca	*P
*Al	*Sn	Pb	V	Cd	*Ni	As
Ti	*Co	Li	Be			
Normal		459	463	489	500	510
516	601	618	633	660	669	729
817	868	887	887	888	894	904
906	916	922	928			
Deficit (D)		459	52	135	152	376
369	280	201	110	87	24	99
0	16	0	0	0	0	0
0	8	0	0			
Excess (E)		11	414	305	277	43
44	48	110	186	182	236	101
112	45	42	42	41	35	25
23	5	7	1			
Imbalance (D+E)		470	466	440	429	419
413	328	311	296	269	260	200
112	61	42	42	41	35	25
23	13	7	1			

Age did not affect TE distribution, but sex did; K, Fe, Al, Pb, Mn, Ni, and Cd were higher in men and Ca, Zn, and Mg in women ($p < 0.05$, two-way ANOVA). The most deficient TE's were (in descending order) Mn, Fe, Se, Zn, and Cr, in women, and Mn, Se, Zn, Fe, and Cr, in men. In women Cr, K, Si, Mg, Cu, and Ca tended to be present both in excess and deficit; whereas in men this was true only for Mg, Cu, and Si. Na and K were found much more in excess then in deficit in both sexes. A cascade of non-essential TE's (Al, Pb, V, Cd, As, Ti, Li, and Be) is evident. In general, the multiple TE imbalances are frequent in Croatia. Hair is a sensitive biological indicator, and nutritional epidemiology may profit from reliable TE hair analysis.

147A.—Retention of Inhaled Particles in the Human Respiratory System and in the Associated Lymphoid Tissue. Teresa Pinheiro,* Luís C. Alves,* Paula Monteiro,[+] M. João Palhano,[+] António Bugalho de Almeida,[+] Fátima Araújo,* Alexandra Barreiros, Sylvain Bohic[++] and Alexander Simionovici.[++]** *Instituto Tecnológico e Nuclear, Sacavém, Portugal, [+]Centro de Estudos de Doenças Pulmonares and Departamento de Pneumologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal,

**Instituto Nacional de Engenharia e Tecnologia Industriais, Lisboa, Portugal, ++ESRF, Grenoble, France.

Information on the composition and on the size distribution of particulate matter deposited along the human respiratory tract can help linking epidemiological, toxicological, and pathological studies and thus potentially improve the understanding of the origin of pulmonary disorders induced by respirable xenobiotics. Along the respiratory tract the composition in chemical elements and the size estimation of inhaled particles was carried out using nuclear microscopy techniques with elemental mapping capabilities which are based on accelerated particle beams (Nuclear Microprobe) and synchrotron radiation (Micro-X Ray Fluorescence Spectrometry). In this work, special emphasis will be given to deposits observed at the lower respiratory tract (small airways and alveoli) and to possible mechanisms of removal to mucosa associated lymphoid tissue (MALT). Cryosections were produced from respiratory and MALT tissue samples taken at autopsy from subjects without pulmonary affections. Particle at the lower respiratory ducts (1 to 5 μm) can be individualised while at lung alveoli particle aggregates are frequent. Metals such as, Cr, Mn, Fe, Ni, Cu, Zn, and Pb can be found in those deposits. Phagocytic cells inclusions, at respiratory tissue and at lymph nodes, are similar, in composition, to particle deposits found at the bronchi mucosa or at alveoli. The mobilisation of elements from deposits to surrounding tissues (bronchi mucosa and pneumocyte cells) or to lymph node cortical areas where lymphoid cells are released is not identical to all chemical elements, pointing out distinct toxicity risks and eventually different underlying biological mechanisms.

148A.—Status of Mg, Fe, Cu and Zn in Brazilian Female Water-polo Team in Pre Competition, Detraining, and Training Periods. Fátima A.A. Sardinha,* Eliane T.L. Mari,* Joao J. Leite and Célia Colli.*** *Fac. of Pharmacy; **Heart Institute, Fac. of Medicine, Univ. of S.Paulo, S.Paulo, Brazil.

The status of Mg, Fe, Cu, and Zn in the Brazilian water polo female team (aged 17–31) was evaluated. Total serum magnesium, copper, and zinc (MgS, FeS, CuS, and ZnS), ferritin (FER) hemoglobin (Hb), % transferrin saturation (%ST) and erythrocyte Mg (er-Mg), Zn (er-Zn) and superoxide dismutase activity (SOD) in sequencial periods of pre competition- Pc ($n = 25$), detraining –Dtr ($n = 8$), and training–Tr ($n = 9$) were determined. The results (medians) showed no significant effects of the training on Mg, Cu and Zn status. On the other hand, in the Pc period the Hb concentration (13.4 g/dL) was significantly higher than that of the Tr one (14 g/dL) ($p < 0,05$), the %ST was higher (28%) than that of the Dtr period (25%) ($p < 0,05$) and so was the SOD activity (4700 U/gHb $\times$ 2500 U/g Hb). Although repeated training bouts of resistence exercise according to many authors may induce acute phase inflammatory response, thus increasing SOD-Er activity, in this study the positive correlation of VO2 max. with SOD activity and er–Mg may be a evidence of the adaptation to exercise. The response of the SOD activity also reflects a good copper status. We conclude that for this group the diet planning foccusing Mg, Fe and Cu status should be a matter of special concern.

Acknowledgments: PRONUT; CAPES, CNPQ; Federação Aquática Paulista; Mario Sérgio Rossi Vieira, MD.

149A.—Comparative Potential of Fruit-root Vegetables with Green Leafy Vegetables and Fruits from India for Antioxidant & Micronutrient Quality. Kirtan Tarwadi and Vaishali Agte. Agharkar Research Institute, G.G. Agarkar Road, Pune-411004, India.

Diets rich in micronutrients and antioxidants have gained increased attention in recent years as natural means to combat oxidative stress. Present paper reports the comparisons of levels of zinc, iron, copper, selenium, manganese and antioxidant capacity in commonly consumed, cooked fruit vegetables (12) and root vegetables (15) with cooked green leafy vegetables (GLV) and fresh fruits previously reported by us. Trace metal levels were estimated by atomic absorption spectrometry. Antioxidant capacity was measured as inhibition of TBARS, super oxide scavenging activity (SOSA) and ferrous iron chelating activity (FICA). Root vegetables as a class showed significantly ($p < 0.05$) higher levels of all the 5 trace metals by 2.5 to 10 times indicating their superiority than fruit vegetables. There were also significant differences ($p < 0.05$) between raw and cooked values of fruit-root vegetables in antioxidant indices. The levels of all the five trace metals were strongly associated with each other within the class of fruit-root vegetables ($p < 0.01$). The antioxidant indices were also associated with each other ($p < 0.05$). However the associations of antioxidant indices with trace metals were marginal within the class of fruit-root vegetables. When compared with the fruits and green leafy vegetables, the trace metal levels were of the order: GLV & root vegetables & fruits > fruit vegetables for zinc and selenium, fruits > roots & GLV > fruit veg. for copper, GLV > root- fruit veg. & fruits for iron, roots & GLV > fruits > fruit veg. for manganese. The inhibition of TBARS was highest in roots (0.59 mM Vitamin E/100g) compared to other classes but raw GLV had higher value (0.73 mM Vitamin E/100) than roots. SOSA values were higher in fruit-root vegetables (36–41mM tannic acid/100 g) than GLV and fruits. FICA was in the range 35–42mM EDTA/100g for fruit-root vegetables and fruits but low in GLV (18.3 42 mM EDTA/100g). As compared to fruits and GLV, vegetables like carrot, turmeric, yams, bitter gourd are rich sources of antioxidants and micronutrients except iron and should be included in diets as promising in defense against oxidative stress and associated debilities.

150A.—Evaluation of Common Foods Recommended for Protein and Mineral Supplementation of Bangladeshi Rice Based Diets. S.H. Thilsted,* K. Kongsbak* and T. Larsen.+ *Research Dept. of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark. +Dept. of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Denmark.

Diets in developing countries are often dominated by a single staple food, rich in carbohydrates but not in protein and minerals. Food based strategies to improve the nutritional status of the population therefore focus on supplementing the diets with small amounts of locally available, commonly consumed

foods with high nutrient densities. In Bangladesh, it is recommended that small amounts of vegetables, legumes and fish should complement the staple, polished rice. The nutritional qualities of two common foods, the leafy vegetable, lal shak (Amaranthus gangeticuss) and the small indigenous fish, mola (Amblypharyngodon mola), both rich in protein and minerals were investigated in the present study. Amaranth and mola fish were included in diets of experimental rats as the only protein, calcium and zinc sources, while other minerals and trace elements were supplied extrinsically. Two protein levels (10% and 16% crude protein) were used in order to achieve different growth rates and weight gains of the rats (a 2 × 2 factorial design). Feed intake, dry matter digestibility, N absorption and body weight gain were dramatically different between diets; higher in the fish diets. Calcium turnover was seriously depressed in the amaranth diets; fractional Ca absorption was app. 5% vs. 30% in the fish diets. The fractional Zn absorption, however, was significantly higher in rats fed the amaranth diets; app. 14% vs. 10% in the fish diets. Similar trends were found in liver tissue and bones; higher Zn contents in rats fed amaranth compared to those fed fish. It can be concluded that the leafy vegetable, Amaranthus gangeticuss is rich in Ca and Zn. However, Ca absorption is greatly depressed, presumably due to inhibitory substances in this vegetable. On the contrary, Zn turnover is unaffected, and thus amaranth can serve as a rich source of bioavailable Zn.

152A.—The Effectiveness of Endemic Goiter Treatment Depends on Some Trace Elements Metabolism Balance in Humans. M.V. Veldanova and A.V. Skalny. Berlin-Chemie (Menarini Group), Moscow, Russia; Center for Biotic Medicine, Moscow, Russia.

According to WHO recommendations the standard treatment of endemic goiter in 10–16 years old children by 100–200 mcg of KI (Berlin Chemie/Menarini Group) during 3–6 months was provided. The beneficial clinical results (decrease or normalization of thyroid gland dimensions) in 66–100% of cases (in different regions) were observed. The average decreasing of thyroid dimensions were 21%. Additionally, we investigated hair concentration of 16 trace elements by ICP-AES and iodine by ionometric method in 295 children. 253 of them (Group 1) demonstrated positive clinical effect, and 42 ones (Group 2) showed the negative results. For teenagers from Group 2 the elevated ($P < 0.05$) hair Mn (2.82 ± 0.47 vs. 0.96 ± 0.1 ppm), Cd (0.35 ± 0.1 vs. 0.11 ± 0.01 ppm), Co (0.18 ± 0.02 vs. 0.12 ± 0.01 ppm) and Ti (0.68 ± 0.11 vs. 0.43 ± 0.4 ppm) concentrations were typical. In contrary, the hair Zn (151.4 ± 6.8 vs. 172.5 ± 4.4 ppm), Se (0.88 ± 0.4 vs. 1.05 ± 0.06 ppm) and Cu (12.3 ± 0.5 vs. 15.6 ± 0.4 ppm) levels were significantly ($P < 0.05$) lower in Group 2. There were no significant differences between the Group 1 and Group 2 in hair Al, As, Cr, Fe, Ni, Si, Sn, V, I, Pb concentrations. So, in this study we suggested the goitrogenic effect of Co, Mn, Cd and, possibly, Ti excesses and Zn, Se, Cu deficiencies. The prophylaxis and treatment of endemic goiter needs the investigation of multifactorial i.e. multielemental nature of this pathologic state, especially in children.

153A.—Dual Fortification of Salt with Iodine and Encapsulated Iron: a Randomized, Double Blind Controlled Trial in Moroccan Schoolchildren. Michael B. Zimmermann,* Christophe Zeder,* Nourredine Chaouki,[+] Amina Saad,[+] Toni Torresani and Richard F. Hurrell.*** *Human Nutrition Laboratory, Swiss Federal Institute of Technology, Zürich, Switzerland; [+]The Ministry of Health, Rabat, Morocco; **Department of Endocrinology, University of Zürich Children's Hospital, Zürich, Switzerland.

Introduction: In many developing countries, children are at high risk for both goiter and iron-deficiency anemia. Iron-deficiency anemia (IDA) adversely effects thyroid and iodine metabolism and reduces efficacy of iodine prophylaxis in areas of endemic goiter. Objective: In a series of studies in northern Morocco, we tested a novel form of dual fortified salt (DFS) containing iodine and encapsulated iron (Fe). Methods: To establish the DFS fortification level, we measured salt intake by 3-day weighed food records and estimated Fe bioavailability from the local diet using published algorithms. We then formulated a DFS containing 25 mg iodine/g salt (as potassium iodide) and 1 mg Fe /g salt (as ferrous sulfate hydrate encapsulated with partially-hydrogenated vegetable oil). After storage and acceptability trials, we compared the efficacy of the DFS to iodized salt (IS) in a 9-month, randomized, double-blind trial in severely iodine-deficient 6–15 yr-old children ($n = 377$) with a high prevalence of iron-deficiency anemia. Results: Salt intake in school-age children was 7–12 g/day, and estimated Fe bioavailability from the local diet was 0.4–4.3%. After storage for 20 weeks, there were no significant differences in iodine content between the DFS and IS, and color stability was acceptable when added to local meals. During the efficacy trial, after 9 months, mean Hb increased by 14 g/L ($p < 0.01$) and serum ferritin, transferrin receptor and zinc protoporphyrin were significantly improved ($p < 0.05$) in the DFS group compared to the IS group. The prevalence of IDA was reduced from 35% to 8% in the DFS group at 9 months ($p < 0.001$). There were no significant differences in median urinary iodine (UI) between the two groups throughout the study. In both groups, from 2 through 9 months, median UI significantly increased compared to baseline ($p < 0.001$) and was above the cut-off value (100 μg/L) for risk of iodine deficiency. At 9 months, the mean decrease in thyroid volume measured by ultrasound in the DFS group (–38%) was twice that of the IS group (–18%) ($p < 0.01$). At 9 months, the goiter rate was significantly decreased in the DFS group compared to the IS group ($p < 0.001$). Mean serum T4 increased significantly from baseline in the DFS group ($p < 0.02$) and was significantly greater than in the IS group at 5 and 9 months ($p < 0.05$). At 5 and 9 months, the prevalence of hypothyroidism (T4 < 65 nmol/L) was significantly reduced in the DFS group compared to the IS group ($p < 0.001$). Discussion: Addition of encapsulated Fe to iodized salt improves the efficacy of iodine in goitrous children with a high prevalence of anemia. A DFS containing iodine and encapsulated Fe can be an effective combination fortification strategy.

154A.—Dietary Biomarkers of Micronutrient Adequacy and Oxidative Stress as Observed in Indian Cataract Patients. Kirtan Tarwadi and Vaishali Agte. Agharkar Research Institute, G.G. Agarkar Road, Pune-411004, India.

Deficiencies of antioxidants and micronutrients as well as chronic exposure to high levels of glucose and galactose have been considered to play key role in the cataractogenesis.

Cataract is one of the major problems in Indians especially after the age of 50 and it leaves the persons to partial visual debilities in later life. Indian diets being cereal based and mainly vegetarian, may lead to micronutrient deficiencies if intakes of protective foods are inadequate. In the present paper, we report the intakes of 40 different commonly consumed foods estimated for 90 nuclear cataract patients of 50 to 70 yr. age and 90 age-sex matched apparently healthy controls through food frequency questionnaire and 24 hour diet recall. Blood status of zinc, iron, copper, selenium and manganese were also estimated using atomic absorption spectrometry at the time of surgery. Plasma zinc and iron levels were positively associated with intake of root vegetables as well as salads and negatively with sweets ($p < 0.05$). Plasma levels of selenium and blood hemoglobin levels had strong positive association with intakes of cereals and animal foods and negatively correlated with sweets ($p < 0.05$). Ceruloplasmin was negatively associated with sweets and positive with tea and salads. Cataract patients had significantly low intake of root-vegetables and salads as compared to controls ($p < 0.05$). Further, the consumption of sweets and animal foods was significantly higher in patients ($p < 0.05$). These food classes may be of value as biomarkers for cataract. However, intake of fruits, milk and milk products, tea, coffee and snacks were comparable within patients and controls. Within the class of cataract patients, lower socio economic (LIG) groups had lower intakes of salads and citrus fruits which are protective foods. Higher income patients had higher intake of animal foods and sweets than their LIG counterparts. These observations coupled with the associations food classes with blood trace metal status indicated different dietary aetiologies in LIG and HIG patients.

155A.—Interaction of Vitamins and Their Active Forms with In Vitro Zinc Uptake by Erythrocytes Under Deficient, Normal and Excess Zinc States. Vaishali Agte, Rashmi Nagmote and Shashi Chiplonkar. Agharkar Research Institute, G.G. Agarkar Road, Pune-411004, India.

Molecular level interactions at post absorptive zinc metabolism have not been well understood but play important role in deciding the biological half life of absorbed zinc. Zinc is required for the stability of erythrocyte membrane and it's protection from oxidative damage and as co-factor in many enzymes such as carbonic anhydrase. It was therefore decided to study the in vitro zinc uptake by human erythrocytes under the range of plasma concentrations representing the zinc deficient (0.35–0.85 ppm), zinc normal (0.85–1.5 ppm) and zinc excess (1.5–2 ppm) states. Interactions at physiological levels of riboflavin, FAD, nicotinic acid, NAD, thiamine, TPP, folic acid and ascorbic acid with in vitro zinc uptake by human erythrocytes were studied at 20 different molar ratios. The zinc uptake curves showed 3 distinct patterns. Rate of zinc uptake over the concentration of zinc varied significantly at these three different states in control tubes as well as in the presence of interactive factors. Under deficient conditions, thiamine and nicotinic acid enhanced the zinc uptakes while TPP; riboflavin and FAD inhibited zinc uptakes. While NAD, folic acid, ascorbic acid did not show any significant effect. The rate of change in zinc uptake over the zinc concentration in deficient state was 1/3 and 2/3 that of normal condition with riboflavin and FAD. Under normal range, riboflavin and FAD continued to act as inhibitors. Under excess zinc conditions, the vitamin–zinc interactions were not significant factors. The rate of zinc uptake was nearly doubled at excess condition than the normal range in controls and in presence of majority of factors. It was 4 times higher in presence of NAD at the excess state. The results indicate role played by thiamine, riboflavin and nicotinic acid in influencing the zinc metabolism of erythrocytes.

156A.—The Effects of Zinc on the Antioxidant Defense System in the Liver of Chicks Exposed to Cadmium. Mirdza R. Apsite and Nadezhda I. Berzina. Institute of Biology of Latvian University, Salaspils, Latvia.

Cadmium is a typical cumulative xenobiotics with long biological half-life in organs of birds as well. Cd acts destructively on all the living cells by mechanisms involving overproduction of reactive oxygen species (1). An increase in resistance of organism cells against the impact of radicals is performed by the multicomponent antioxidant protection system, which includes Zn. The aim of this work was to investigate the action of Zn in antioxidative processes of protection after introduction of Cd in chicks. For the experiment 1–35 days old Lohman Brown cockerels were used. The chicks were divided into three groups: 1) chicks of the control group received full-value combined food (CF); 2) chicks of the second group individually were administered per os solution CdCl2 (500 μg Cd per 100 g body mass) every day starting from the 20th day of experiment during the next two weeks (+Cd); 3) chicks received CF with elevated level of Zn (500 mg Zn per kg food) + CdCl2 from the 20th day like chicks of the second group (+Cd+Zn). Cd and Zn in liver were estimated by atomic absorption spectrophotometry. Se was estimated by fluorometry. Lipid peroxidation activity was estimated by methods described in (2). Toxicity of Cd was manifested by increased the amount of malondialdehyde (MDA) in liver by ten times in comparison with the level in control chicks, by lowered concentration glutathione in liver (by 16%), by retarded glutathionperoxidase (GSH.Px) activity to a level of 77.3% of its activity in chicks blood of the control group, by lowered Se concentration in liver by 29.4%, however, the accumulation of Zn had a tendency to increase. In control chicks Cd concentration was 0.017 mg/g of liver ash, in +Cd chicks–1.418 mg/g. Addition of Zn to the diet of chicks exposed to Cd reduced the MDA production in liver (the level of MDA was much about the same as in control), enhanced the GSH.Px activity in blood by 9.1%, significantly increased the concentration of Se by 30% and minimized Cd concentration in liver of chicks (+Cd+Zn).

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157A.—Decrease of Free-choice Ethanol Consumption in Ethanol Dependent Rats through Daily Supplementation of Zinc and Copper Amino Acid Chelates. Ashmead H.D., Graff D.J. and Ashmead S.D. Albion Advanced Nutrition, Clearfield, Utah; Weber State University, Ogden, Utah; Albion Advanced Nutrition, Clearfield, Utah.

Ethanol abuse exacerbates copper and zinc deficiencies. This precipitates alterations in ethanol metabolism, which in turn frequently leads to increased ethanol intake. The purpose of this study was to investigate the effects of oral supplementation of bioavailable copper and zinc amino acid chelates on ethanol intake in ethanol dependent rats compared to control rats. After creating ethanol dependency in both groups, each animal in the treated group was gavaged daily with 100- μg Zn and 45- μg Cu/animal/day as amino acid chelates for 21 days. All animals in both groups had free access to both water and a 5% ethanol/95% water mixture (v/v) throughout the study period. The group receiving copper and zinc amino acid chelates voluntarily restricted its ethanol intake and increased its water consumption compared to unsupplemented control animals as seen in Figures 1 and 2. The results were highly significant ($p < 0.001$). When administered as amino acid chelates, copper and zinc appear to reduce the physical need for ethanol in ethanol dependent subjects.

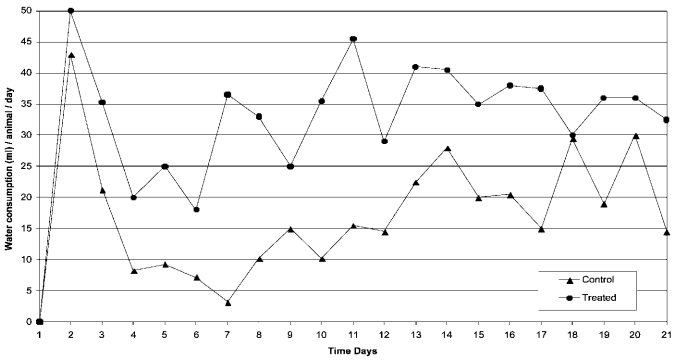

FIGURE 1 Mean water consumption/animal/day.

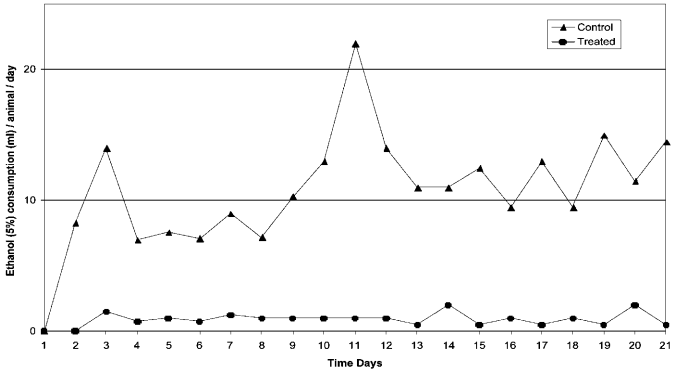

FIGURE 2 Mean ethanol consumption/animal/day.

158A.—Zinc Nutriture and its Bioavailability in Rural Guatemalan Children. Raquel Campos,* Manolo Mazariegos,* Brenda Barahona,* Noel W. Solomons,* Victor Raboy,[+] John Dorsch,[+] Nancy Krebs, Jamie Westcott** and Michael Hambidge.**** *Center for Studies of Sensory, Impairments, Aging and Metabolism (CeSSIAM), 01011, Guatemala City; [+]USDA/ARS National Small Grains Germplasm Research Facility, Aberdeen, 83210, Idaho, USA; **Section of Nutrition, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, 80262, CO, USA.

Zinc nutritional status in developing countries is still of great concern as a public health problem given its impact on linear growth and morbidity in children under five. Usually zinc intakes are low and frequently co-exist with a high intake of absorption-inhibitors, especially phytates and calcium. Maize, the staple food in MesoAmerica, has a very high phytate content, and is believed to play an important role in determining zinc nutritional status in these populations. As a part of a nutritional metabolic study in a rural village near Guatemala City, using a new phytate-reduced maize variety, we studied the baseline dietary intake of a group of 40 children (7–11 y) participating in a zinc availability protocol. Dietary intake assessment was carried out by using a seven-day food frequency questionnaire, and nutrient intake was estimated by adapting food composition data from the World Food System 2 (University of California, Berkeley, CA, USA). Findings for daily intakes among these children were: energy, 1807 ± 510 kcal; total protein, 56.5 ± 16.0 g; animal protein, 16.4 ± 11.2 g; Zn, 0.13 ± 0.04 mmol; Ca, 22.7 ± 8.6 mmol; phytate, 3.87 ± 1.05 mmol. In terms of contribution to the habitual diet, nixtamalized maize provided 46.3 ± 14.3% of energy; 42.4 ± 14.5% of Zn; 70.5 ± 16.4% of Ca and 67.8 ± 12.1% of phytate. The average phytate/Zn millimolar ratio was 31.7 ± 7.5, whereas that for Ca x phytate/Zn per MJ ratio was 94.9 ± 32.2. Conclusion: This study confirms that maize consumption in this population contributes significantly to energy intake, but also is the principal source of mineral absorption inhibitors. The profile presented is typical of a diet with poor mineral availability. Efforts to reduce the load of inhibitors in the diet should have a greater impact on the nutrition status of populations at risk. Mineral nutrition interventions (supplementation and fortification) should also take into account the poor bioavailability in the diet of these populations.

Funded by The Thrasher Research Fund, USA and IAEA, Austria.

159A.—Zinc Homeostasis and Teratogenicity in Normal and Metallothionein-null Mice: Comparing the Effects of Ethanol and Lipopolysaccharide. Luke C. Carey,*† Peter Coyle,* Paulien Berbée,* Jeff C. Philcox* and Allan M. Rofe.* *Division of Clinical Biochemistry, Institute of Medical and Veterinary Science, Adelaide 5000 Australia; †Department of Physiology, University of Adelaide, Adelaide 5000 Australia.

The teratogenic nature of ethanol (EtOH) is thought to be in part mediated by a decrease in fetal supply of Zn, a trace element crucial for normal development. EtOH induces the Zn binding protein metallothionein (MT) in the maternal liver, which in turn sequesters Zn from the plasma, and decreases the plasma Zn concentration. We have previously shown in mice that these changes correlate with decreased transfer of Zn to the fetus, and consequent increased teratology as assessed in late gestation (Carey et al, 2000). Mice that do not express MT (MT−/−) are protected from these effects. This study was designed to assess the effect of lipopolysaccharide (LPS, a potent inducer of MT) on maternal-fetal Zn homeostasis and resultant teratology, and to compare the findings with those obtained previously using EtOH. Non pregnant normal (MT+/+), and MT−/− mice were treated with LPS (0.5 g/g, subcutaneous) or 25% EtOH (0.015 mL/g, intraperitoneal) and killed over 16 h. At 16 h post injection, liver MT levels were increased by approximately 25 fold and 10 fold in LPS and EtOH treated mice respectively.

Plasma Zn concentrations decreased from normal (13 mol/L) by 80% at 6 h in LPS, and by 66% in EtOH treated mice. MT+/+ and MT−/− mice were injected with LPS or EtOH on day 8 of gestation, and killed on day 18. Fetuses from MT+/+ dams exposed to LPS or EtOH exhibited significant teratology: 32% and 28% respectively were abnormal, whereas those from MT−/− dams were relatively unaffected at less than 7% for each treatment, a level similar to that seen in saline treated control mice. These findings indicate that: (1) LPS exerts profound effects on hepatic MT expression and Zn homeostasis in a manner which is similar to that observed with EtOH, (2) like EtOH, the teratogenic nature of LPS appears to be linked to these changes in materno-fetal Zn homeostasis, as is emphasized by the resistance of MT−/− fetuses to this teratogenicity.

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160A.—Effect of Feeding Organic and Inorganic Sources of Additional Zinc on Growth Performance and Zinc Balance in Nursery Pigs. Chanda L. Case and Marcia S. Carlson. University of Missouri, Columbia, MO.

Three experiments were conducted to evaluate the effect of feeding pharmacological concentrations of zinc (Zn), from organic and inorganic trace mineral sources, on growth performance, plasma and tissue Zn accumulation, and Zn excretion of nursery pigs. Blood from all pigs was collected for plasma Zn determination on d 14 in Experiment 1 (Exp. 1), d 7 and 28 in Experiment 2 (Exp. 2), and d 15 in Experiment 3 (Exp. 3). In Exp. 1, 2 and 3, 90, 100 and 15 crossbred (GenetiPorc USA, LLC, Morris, MN) pigs were weaned at 24 ± 0.5, 18, and 17 d of age (6.45, 5.47, and 5.3 kg avg. initial BW), respectively, and allotted to dietary treatment based on initial weight, gender, and litter. A Phase 1 nursery diet was fed as crumbles from d 0 to 14 in Exp. 1, 2, and 3, and Phase 2 nursery diet was fed as pellets from d 15 to 28 in Exp. 1 and 2. The Phase 1 and Phase 2 basal diets were supplemented with 100 ppm Zn as ZnSO4. Both dietary phases contained the same five dietary treatments; 150 ppm additional Zn as zinc oxide (ZnO), 500 ppm added Zn as ZnO, 500 ppm added Zn as a Zn-amino acid complex (Availa-Zn 100), 500 ppm added Zn as a Zn-polysaccharide complex (SQM-Zn), and 3,000 ppm added Zn as ZnO. Overall in Exp. 1, pigs fed 500 ppm added Zn as SQM-Zn or 3,000 ppm added Zn as ZnO had greater ADG ($P < 0.05$) than pigs fed 150 ppm or 500 ppm added Zn as ZnO, or 500 ppm added Zn as Availa-Zn 100 (0.44 and 0.46 kg/d vs. 0.35, 0.38, and 0.33 kg/d respectively). Overall in Exp. 2, pigs fed 3,000 ppm added Zn as ZnO had greater ($P < 0.05$) ADG and ADFI than pigs fed any other dietary treatment. On d 14 of Exp. 1, and d 28 of Exp. 2, pigs fed 3,000 ppm added Zn as ZnO had higher ($P < 0.05$) plasma Zn concentrations than pigs on any other treatment. In Exp. 3, fecal, urinary, and liver Zn concentrations were greatest ($P < 0.05$) in pigs fed 3,000 ppm added Zn as ZnO. On d 10 to 15 of Exp. 3, pigs fed 3,000 ppm added Zn as ZnO had the most negative Zn balance ($P < 0.05$) compared with pigs fed the other four dietary Zn treatments. In conclusion, feeding 3,000 ppm added Zn as ZnO improves nursery pig performance; however, under certain nursery conditions the use of 500 ppm added Zn as SQM-Zn may also enhance performance. The major factor affecting nutrient excretion appears to be dietary concentration, independent of source.

161A.—Zinc Inhibition of Hepatic Gluconeogenesis and Lactate Production from Fructose. Peter Coyle, Evelien M. Tichelman, Rinske T. Pauw, Jeffrey C. Philcox and Allan. M. Rofe. Division of Clinical Biochemistry, Institute of Medical and Veterinary Science, Adelaide, SA 5000 Australia.

Zn is required for activity of over 300 enzymes including many involved in carbohydrate metabolism. Zn may act as an allosteric regulator of carbohydrate metabolism in vivo, as metabolic activity has been found to be associated with changes in intracellular free Zn (1). Gluconeogenesis is sensitive to Zn, and liver fructose 1,6-bisphosphatase has been found to possesses both a high affinity binding site for Zn activation, and a lower-affinity site where Zn inhibits the enzyme (2). Fructose is a common gluconeogenic precursor in mammals and its rapid conversion to glucose and glycogen in the liver has been widely studied. The ability of Zn to modulate key metabolic processes was investigated in a study of gluconeogenesis in isolated hepatocytes from fasted rats. The rate of gluconeogenesis from 20 mM substrate was greatest in the order fructose = sorbitol > glycerol > glyceraldehyde. Zn (100 M) inhibited glucose production from fructose by 41%, sorbitol, 28%; glycerol 17%; and glyceraldehyde, 26%. Maximum inhibition of gluconeogenesis from fructose occurred at 25 M Zn. Lactate production was greatest in the order, glyceraldehyde > fructose > sorbitol > glycerol. Zn inhibited the rate of lactate production from fructose by 24% but not from any other substrate. The electron acceptor, phenazine methosulphate (PMS), doubled the rate of lactate production from sorbitol and this rate was inhibited 11% by Zn. A strong positive linear relationship ($r = 0.994$) was obtained between inhibition by Zn of glucose and lactate production indicating that a common pathway is inhibited by Zn. The inhibition of fructosebisphosphatase by Zn is well recognized. However in studies with semi-purified rat liver enzyme preparations we found that Zn also inhibited fructokinase and aldolase-B which are common enzymes in fructose metabolism to glucose and lactate. The possibility that Zn might effect the cellular uptake of fructose is also being investigated.

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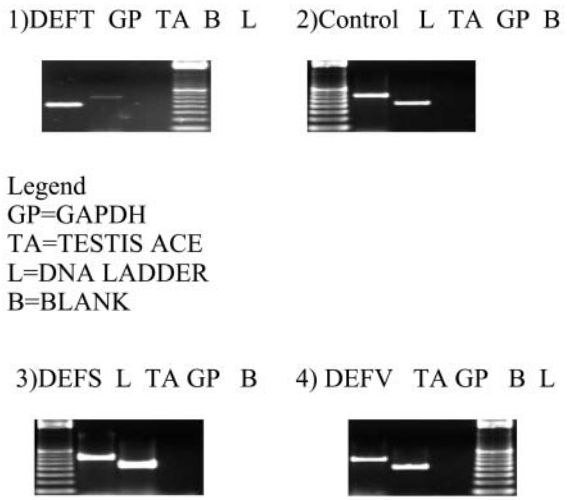
162A.—Zinc Repletion Administered in Different Ages to Zinc Deficient Rats can Significantly Change the Activity and the mRNA Expression of Testis Angiotensin-converting Enzyme. G.S. Henriques,* A.G.H. Silva,* H. Hirata[+] and S.M.F. Cozzolino.* *Pharmaceutical Sciences Faculty–Department of Food Science and Experimental Nutrition–University of Sao Paulo–Brazil; [+]Pharnaceutical Sciences Faculty–Department of Clinical Analysis and Toxicology–University of Sao Paulo–Brazil.

The testicular isoform of Angiotensin Converting Enzyme coordinates one atom of zinc in its site of coordination and can serve as a biomarker to this metal. This study aimed to quantify the activity and the expression of mRNA to ACE in testis of zinc depleted rats at breast-feeding period and after supplemented with zinc in different ages. For this purpose, 8 Wistar (Rattus norvegicus) pregnant females were housed in individual cages and they had ad libitum access to a zinc deficient diet (AIN-93 DZn). They also had free access to pure deionized water. At the end of breast-feeding period (21 days), 32 male pups were weaned and selected in 4 experimental groups (8 per group) housed in individual cages. They had free access to diets with different concentrations of zinc–DEFT (rats fed with 8,5 mg of Zn/Kg of diet until killed at 22nd day), Control (rats fed with 35 mg of Zn/Kg of diet until killed at 22nd day), DEFS (rats fed with 8,5 mg of Zn/Kg of diet until 22nd day and then supplemented with 350 mg Zn/Kg of diet, killed at 50th day) and DEFV (rats fed with 8,5 mg of Zn/Kg of diet until 28th day and then supplemented with 350 mg Zn/Kg of diet, killed at 50th day). After each period, the animals were killed and testis were removed to determine ACE activity by colorimetric assay and testis ACE mRNA expression by RT-PCR techniques. Total zinc content in testis was determined by FAAS. The means obtained for ACE testis activity were 260,96 ± 22,58 for DEFT, 391,04 ± 21,60 for Control, 614,20 ± 27,05 for DEFS and 525,74 ± 26,45 for DEFV (all mmoles of Gly-Gly/min. Kg tissue). Zinc concentrations in testis were 20,12 ± 1,43; 22,29 ± 0,88; 25,83 ± 0,71 and 26,26 ± 1,60 mg/g of tissue for DEFT, Control, DEFS and DEFT respectively. Correlation analysis between ACE testis activity and expression of his mRNA showed that rats which were fed with zinc supplemented diets, after an while (DEFV), had a significant decreased in those enzyme parameters, suggesting an impairment in replace zinc normal status in testis of post-pubertal rats. Supported by FAPESP.



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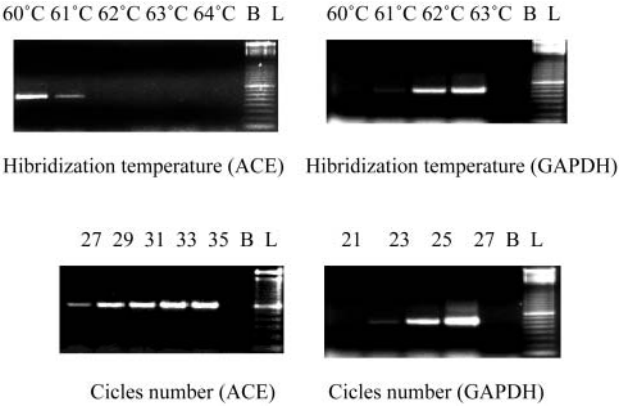
163A.—Optimization of the RT-PCR Technique for the Determination of the mRNA Expression to Testis Angiotensis-converting Enzyme in Zinc Treated Rats. G.S. Henriques,* A.G.H. Silva,[+] M.H. Hirata[+] and S.M.F. Cozzolino.* *Pharmaceutical Sciences Faculty – Department of Food Science and Experimental Nutrition – University of Sao Paulo- Brazil; [+]Pharnaceutical Sciences Faculty – Department of Clinical Analysis and Toxicology – University of Sao Paulo–Brazil.

Reverse transcriptase and polimerase chain reaction (RT-PCR) are powerful and widely used techniques to measure gene expression. The objective of the present work was to optimize reactional conditions that could generate large variability and introduce sistematic errors in polimerase chain reaction. Hybridization temperatures and number of cicles of denaturation, hybridization and extension were matched. For this purpose, samples of rat testis were obtained from a trial in which a zinc supplementation diet was used. For the extraction of total RNA, fenol-cloroform-isotiocyanate reaction was used. After that, stable cDNA was generated by reverse transcriptase and it served as template for PCR proves. Two sets of primers were used, one for the expression of testis angiotensin-converting enzyme and the other for the expression of GAPDH housekeeping gene. PCR products were submitted to an eletrophoresis in an 1,5% agarose gel for 35 minutes at 100 volts, stained by ethidium bromide and then visualized at UV chamber. Results showed that the best temperature of hybridization for target gene (ACE) and standard housekeeping gene (GAPDH) were quite different, 60 and 62 °C respectively. The same tendency occurred with the PCR's cicles, 31 and 25 cicles respectively. We concluded that with this optimization and adjustments in other variables (e.g. DNTP and MgCl2 concentration) we could minimize interferences in the technique, laying to obtain true and comparative data for testis ACE gene expression. Supported by FAPESP.



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164A.—Assessment of the Risk of Zinc Deficiency in Mexican Preschoolers by Dietary Intakes of Absorbable Zinc and Serum Zinc Concentration. Christine Hotz and Juan A. Rivera. Instituto Nacional de Salud Publica, Cuernavaca, Morelos, Mexico.

Much information has accumulated as to the possible widespread occurrence of zinc deficiency, however, population-based assessments of the prevalence of zinc deficiency in at-risk countries are lacking. The Mexican population may be at elevated risk of zinc deficiency as the largely maize-based diet contains large amounts of zinc absorption inhibitors (e.g. phytate, calcium). Data from the Mexican National Nutrition Probabilistic Survey (1999) were used to estimate the risk of zinc deficiency among non-breastfed preschool children (1–4 years) using both serum zinc concentration (sZn; n = 124) and the estimated amount of bioavailable dietary zinc (Abs Zn) determined from a single 24-hr recall per child (n = 1016). The amount of Abs Zn was estimated based on the total zinc, protein, calcium, and phytate content of the diet (IZiNCG, unpublished), calculated on a per meal basis and summed for total day's intakes. The prevalence of inadequate zinc intakes was determined from the estimated proportion of children with Abs Zn below the WHO requirement (0.9 mg/d), assuming the CV of the population intake distribution is 25%. Prevalence of zinc deficiency determined by sZn was calculated as the proportion of children with sZn <65 ug/dL. Both Abs Zn and sZn differed significantly by Region (North, Central, South) and Socioeconomic status (SES; High, Low, Moderate) group (ANOVA; $p < 0.05$). The predicted prevalence of zinc deficiency from low Abs Zn and low sZn for the 3 Regions and 3 SES groups were:

	North	Central	South	High SES	Mod SES	Low SES
Abs Zn	48%	57%	79%	25%	54%	95%
sZN	18%	35%	46%	21%	25%	48%

The Regional and SES group trends in relative risk of zinc deficiency followed expected patterns of nutritional adequacy among these groups. Similar trends in relative risk of zinc deficiency were apparent when using dietary and biochemical assessment methods, although the absolute prevalence of risk was higher using Abs Zn than with sZn. Although the accuracy of these methods to identify zinc deficiency in individuals cannot be ascertained, either assessment method may be useful to identify differences in the relative risk of zinc deficiency at the group level.

165A.—Zinc Homeostasis among Children on Bed Rest after Bone Trauma. Christine Hotz,* K. Michael Hambidge,† Nancy F. Krebs,† Jamie E. Westcott,† Rosalind S. Gibson* and Mark J. Manary.‡ *University of Otago, Dunedin, New Zealand, †University of Colorado, Denver, Colorado, USA, ‡Washington University, St Louis, Missouri, USA.

Pilot data are presented from a stable isotope study measuring absorption and retention of zinc among Malawian children on bed rest during recovery from bone trauma. Subjects on bedrest have previously demonstrated negative zinc balances. However, unlike traditional balance studies, isotope tracer studies can be used to elucidate the points of regulation of zinc homeostasis that contribute to negative balance. Subjects 3–14 y of age (n = 16) were recruited from an urban Malawian hospital. Bedrest subjects included 3 children recovering in traction 1 week following femur fracture and 4 children within the 2nd-6th week of recovery on bedrest from pelvic or leg fractures and walking only as needed. Control subjects (n = 9) were relatively well inpatients of well-siblings residing at the hospital. The study diet was a maize:soy porridge providing 0.3 and 0.4 mg Zn/kg/d in the bedrest and control groups, respectively. Children received an intravenous dose of 70Zn, and an oral dose of 67Zn was divided and given with each of 5 meals (Day 0). Urine samples were collected between days 3 and 8 and the enrichment ratio of 67Zn and 70Zn was used to calculate fractional absorption of Zn (FAZ). Endogenous fecal zinc (EFZ) was calculated by isotope dilution method from urine and fecal collections made between Days 4 and 8. Bedrest children had a significantly greater loss of EFZ compared to control children (3.1 vs 1.5 mg/d; $p < 0.05$; ANCOVA, controlling for body weight). They also had significantly lower FAZ (0.14 vs 0.24), and therefore a lower amount of total absorbed zinc (TAZ; 1.2 vs 1.8 mg/d) and net absorption of zinc (−1.9 vs 0.2 mg/d) compared to controls ($p < 0.05$; ANCOVA, controlling for body weight). Both increased EFZ and decreased FAZ contributed to the negative zinc balance observed among the bedrest subjects. Despite the excess losses of intestinal endogenous zinc, likely related to bone resorption/muscle atrophy during bedrest, a compensatory response of increased absorption of exogenous zinc was not observed, suggesting a perturbation in homeostatic regulation. Further study is needed to elucidate the mechanisms involved in these regulatory changes and whether chronic bedrest conditions may lead to compromised zinc status in vulnerable populations.

166A.—Functional Characterization of a Novel Mammalian Zinc Transporter, ZnT5. Liping Huang,*+ Catherine P. Kirschke* and Jane Gitschier.** *Western Human Nutrition Research Center/ARS/USDA and +Department of Nutrition, University of California Davis, Davis, CA 95616, USA and **University of California San Francisco, San Francisco, CA 94143, USA.

Introduction: Zinc is an essential trace element required for the structural stability of a variety of proteins as well as for the catalytic activity of metallo-enzymes. Two families of zinc-transporter proteins, ZnT (Zinc Transporter) and ZIP (ZRT1, IRT1-like protein) have been identified in mammals. The ZnT proteins, which are the members of the CDF family (cation diffusion facilitator), appear to function either by transporting zinc out of cells or by sequestering zinc into intracellular compartments. In contrast, the ZIP proteins appear to function in uptake of zinc into the cytoplasm. Methods: We used northern and western blot analysis, immunofluorescent staining, and functional analysis of yeast mutants of zinc transporters to illustrate the function of a novel mammalian zinc transporter. Results: We characterized the mammalian ZnT5 protein, a new member of the CDF family of heavy metal transporters. The human ZNT5 gene was mapped at 2p21–22, while the mouse Znt5 was localized to chromosome 17. Overexpression of ZnT5 in both wild-type yeast and mutants that are deficient in cytoplasmic zinc causes growth inhibition, but this inhibition is abolished in mutant cells with high cytoplasmic zinc. ZnT5 may function in transporting the cytoplasmic zinc into the Golgi apparatus as well as the vesicular compartment, as evidenced by its overlapping intracellular localization with TGN38 and transferrin receptor (TfR) in the normal rat kidney (NRK)

cells. We also demonstrate that the intracellular distributions of ZnT5 as well as ZnT4 are regulated by zinc in the NRK cells. The results from this report, combined with those from other studies, suggest that the intracellular zinc homeostasis is mediated by many ZnT proteins, which act in tissue-, cell-, and organelle-specific manners.

167A.—Plasma Zinc and Body Fat Mass in Brazilian Elite Athletes. Koury, J.C.,* Portella, E.S.,* Oliveira, A.V.,[+] Oliveira, C.F.,[+] Bogea, C.P.* and Donangelo, C.M.** *Instituto de Nutrição – Universidade do Estado do Rio de Janeiro UERJ; [+]Instituto de Educação Física e dos Desportos – UERJ; **Instituto de Química – Universidade Federal do Rio de Janeiro – Brasil.

There are limited data on the relationship between zinc status, body composition, control of body weight and physical activity. In this study, we compared plasma zinc levels, body composition, energy and zinc dietary intake, and their relationship in Brazilian elite athletes of three anaerobic modalities: swimmers (n = 13), runners (n = 9) and judoists (n = 7). All athletes gave informed consent before participation in the study that was approved by the Ethical Committee of Universidade do Estado do Rio de Janeiro. Fasting blood samples were obtained 24 hours after competition. Dietary intake was assessed by 24 hours recall and analyzed using the NutriSurvey database. Plasma zinc was determined by atomic absorption spectrometry. Percent body fat was evaluated based on seven skin-fold measurements according to the Pollock protocol. Statistical comparison between modalities were done using one-way ANOVA. Associations between variables were examined by Pearson correlation analysis after application of the Kolmogorov -Smirnov test. Energy and zinc dietary intakes were similar in the three athletic modalities, on average, 3786 ± 457 Kcal/day and 15 ± 5 mg / day, respectively. Compared to the theoretical energy needs, dietary energy intake was higher for swimmers (9%) and lower for runners (29%) and judoists (12%). Plasma zinc was similar in the three modalities, on average 119 ± 20 μg/dl. Total body weight was also similar in the three modalities (75 ± 8 kg). Percent body fat was higher ($p < 0,05$) in swimmers (8 ± 2%) than in runners (5 ± 1%) but similar to judoists (7 ± 2%). There was no significant correlation between percent body fat and energy or zinc dietary intakes of the athletes. In swimmers, percent body fat was inversely associated with plasma zinc ($r = -0,60$; $p = 0,03$). Our results suggest the control of body weight and of body fat mass is very efficient in elite athletes of anaerobic modalities. In swimmers, this control is probably related to an interaction between plasma zinc and leptin secretion.

168A.—Cardiac Neural Crest Cells are Impaired by Dietary Zinc Deficiency; a Mechanism for Developmental Heart Defects in Rat Fetuses. Louise Lanoue,* Veronica Lopez* and Carl L. Keen.*[+] Departments of *Nutrition and [+]Internal Medicine, University of California at Davis, Davis, CA.

Heart defects represent the most frequent type of human congenital malformations accounting for nearly one third of all birth defects. Results from a previous study show that 82% of gestation day (GD) 20 fetuses from dams fed a zinc (Zn) deficient diet exhibited cardiac anomalies compared to 2.2% of the Zn adequate fetuses; a large proportion of the malformations indicated that neural crest cell (NCC) metabolism may have been affected by Zn deficiency. To test the hypothesis that an abnormal cardiac NCC metabolism resulting from Zn deficiency is a factor that contributes to heart developmental defects, we collected GD 11, 13 15 and 18 rat fetuses from dams fed either a Zn adequate (25 mg Zn/g) or a Zn deficient (0.5 ug Zn/g) diet, or pair fed the control diet in amounts equal to those consumed by dams fed the Zn deficient diet. Histological examination of the developing hearts of GD 13 and 15 rat fetuses show increased incidence of anomalies in the Zn deficent group such as incomplete septation of the ventricles, abnormal valve formation, decreased trabeculae, and thin ventricular wall. Analysis of cardiac NCC migratory pathway using the HNK-1 antibody showed positively stained cells restricted to the interventricular septum (GD 11 and 13), and to the wall of the outflow tract (GD 15), while smooth muscle cell actin was uniformly expressed throughout the myocardium, in all groups. Compared to control, GD 13 Zn deficient embryos had increased cell death in the somites, visceral arches and in the inter-ventricular septum of the heart as assessed by TUNEL. The observation that TUNEL stained cells co-localized with HNK-1 positive cells suggest that cardiac NCC may be susceptible to Zn deficiency and that cell death may be one mechanism contributing to the Zn-deficiency induced heart anomalies. Supported by the Clinical Nutrition Research Unit of UC Davis (CNRU-DK35747).

169A.—Accumulation of Zinc in N-methyl-N-nitrosourea-induced Mammary Tumors in Rats. Ricky Lee, Helene Duminy, Wendy Woo and Zhaoming Xu. Food, Nutrition and Health Group, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

Some human studies have showed an accumulation of zinc in the breast cancer tissues while other human studies have showed that zinc concentration in breast cancer tissues is either unchanged or decreased. N-methyl-N-nitrosourea-induced rat mammary tumorigenesis is a widely used rodent model for studying breast cancer because they share many similarities in hormone dependency, pathogenesis, histological classification and immunocytochemical markers. The objective of this study was to establish if there is an accumulation of zinc in N-methyl-N-nitrosourea-induced mammary tumors. Female Sprague-Dawley rats were either sham-treated or N-methyl-N-nitrosourea-treated (50 mg/kg BW) for 100 days. On a per dry weight basis, zinc concentration in mammary tumors was 11 times of that in normal mammary glands and 9 times of that in tumor-free mammary glands. On a per protein basis, zinc concentration in mammary tumors was 1.4 and 1.3 times of that in normal mammary glands and tumor-free mammary glands. Methallothionein level in mammary tumors was 9 and 10 times of that in normal and tumor-free mammary glands, respectively. Zinc concentration and methallothioneine level were the same between normal and tumor-free mammary glands. RT-PCR analysis revealed an increased mRNA level of Nramp-2, a divalent metal transporter, in mammary tumors comparing with the level in mammary glands. In summary, these results showed a zinc accumulation in N-methyl-N-nitrosourea-in-

duced rat mammary tumors, possibly via an increased zinc import.

170A.—Zn Nutriture Assessment using Dietary Intake and Biochemical Indicators among South Koreans: Focusing on Sex and Area Difference. Soo-Lim Lee, Eun-Jung Whang, Eun-Kyung Shin, Chong-Suk Kwon, Mal-Soon Ha,[+] Baek-Il Kim,[++] Jin-Sook Yoon[+++] and In-Sook Kwun.* *Department of Food Science and Nutrition, Andong National University, Andong, [+]Andong Public Center, [++]Andong Medical Center, [+++]Department of Food Science and Nutrition, Kyemyung University, Taegu, South Korea.

Zn has been known as one of the antioxidant trace elements and marginal Zn deficiency is general in public health. Zn nutritional status in South Korea, using Zn, Ca and phytate intakes, phytate: Zn molar and phytate x Ca: Zn millimolar ratios, was evaluated in previous study. At the present study, Zn nutriture assessment in South Koreans was evaluated, specially focusing on sex difference in rural, urban and metropolitan areas. Random sample of 625 subjects aged between 20 and 70 yrs were selected for the current study. Food frequency questionnaire (FFQ) was used for the estimation of nutrient intakes and 24-hour dietary recall was used for validity of FFQ. Blood, urine, hair and nail were collected for Zn analysis and alkaline phosphatase (ALP) activity was also measured. Average Zn intake (mean ± SD) was lower in the rural (6.5 ± 3.7 mg/d) and the urban area (7.3 ± 4.4 mg/d) than in the metropolitan city (11.4 ± 3.1 mg/d) ($p < 0.05$). Mean Zn intake of Koreans is lower than the Korean RDA for adults (12 mg/d for man and 10 mg/d for woman). There is no statistical difference between men and women in zinc intake in all three different areas. Other nutrient intakes and molar ratios which can affect on the Zn nutriture, such as Ca, phytate, phytate: Zn, and phytate x Ca: Zn, were not different between men and women in rural, urban and metropolitan areas, except molar ratio of phytate: Zn in rural area was higher in women than in men. Plasma and urinary Zn concentration was also not different between men and women in three different areas, but red blood cell Zn concentration was higher in women (8.9 ± 2.1 μg/mL) than in men (7.7 ± 1.4 μg/mL) ($p < 0.05$) in metropolitan city area. The results of the present study showed that the suboptimal zinc nutriture among South Koreans, but there is not much difference in zinc nutriture between the different sex in different areas. (Supported by the Korean Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea, HMP-00-B-22000-0040)

171A.—Molecular Roles of Zinc After Brain and Neuronal Injury. Cathy W. Levenson, Jacob W. Van Landingham, E. Carden Yeiser and Jerrod P. Libonati. Program in Neuroscience, Florida State University, Tallahassee, Florida, USA.

To study both the long and short-term molecular roles of zinc (Zn) after brain and neuronal injury, we have developed models of traumatic brain injury (TBI), ischemic injury, and copper-induced neuronal injury. Northern analysis, in situ hybridization and immunhistochemistry showed that 4 weeks after TBI, expression of the zinc-binding protein metallothionein-3 (MT-3) increased in and around the site of injury. This increase was evident only if the injury occurred in adult animals. Injury in rats on postnatal day 1–3 resulted in no increases in MT-3. Moderate Zn deficiency, often seen clinically after human TBI, exacerbated secondary cell death measured by TUNEL staining at the site of injury. Co-localization of TUNEL staining with cell-specific markers revealed that Zn deficiency increased DNA damage in ED-1 and OX-42 positive macrophages/microglia that participate in debris clearance, suggesting a role for Zn in repair processes after brain injury. Caspase activity, nuclear morphology, p53 expression and nuclear localization, and annexin V staining were used to show that injury to post-mitotic cultured human neurons (NT2-N) using either 100 μM cobalt (to mimic ischemia) or copper (to mimic neuronal damage in Wilsons Disease) resulted in apoptosis. Dependence of apoptosis on the tumor suppressor protein p53 was established by transfection with a dominate-negative p53 construct that eliminated both p53 expression and copper-mediated death. Furthermore, the addition of Zn to both models of neuronal injury prevented apoptosis by induction of the protective chaperone protein Hsp 70 and the prevention of p53 nuclear translocation.

172A.—Zn^{2+} Upregulates Metallothionein and Reduces NFkB and AP1 Activity in Pancreatic Islets Ex Vivo and Prevents Diabetes Induced with Multiple Low Doses of Streptozotocin in Mice. Abdelhakim Lgssiar, Mohamed Hassan, Patricia Schott-Ohly, Nadira Friesen and Helga Gleichmann. German Diabetes Research Institute, Düsseldorf, Germany.

Diabetes can be induced with multiple low doses of streptozotocin (MLD-STZ) in susceptible strains of male mice. Both toxicity on the essential b-cell structure glucose transporter 2 (GLUT 2) and inflammatory immune reactions are involved in the pathogenic path-way. Treatment with Zn^{2+}-enriched drinking water upregulated metallothionein (MT) in islets and prevented MLD-STZ diabetes (1). By analyzing the mechanism underlying the protective effect mediated by Zn^{2+}, we asked, whether upregulated MT scavenged MLD-STZ-stimulated generation of ΣOH and thereby prevented MLD-STZ-triggered NFkB and AP1 activation as transcripton factors for inflammatory cytokine production. In vitro, STZ stimulated ΣOH generation in isolated islets as determined by ESR spectroscopy (2). Zn^{2+}-enriched drinking water significantly reduced basal NFkB and AP1 activation in isolated islets as determined by EMSA. MLD-STZ, in contrast, significantly induced activation of these transcription factors. Treatment with Zn^{2+}-enriched drinking water, however, significantly reduced MLD-STZ-induced activation of NFkB and AP1. In conclusion, Zn^{2+}-upregulated MT may scavenge MLD-STZ-stimulated ΣOH generation, which, in turn, may prevent NFkB and AP1 activation. These effects may inhibit stimulation of proinflammatory cytokine production in the micromilieu of pancreatic islets and rescue b-cells from destruction induced by MLD-STZ. Since inflammatory immune reactions are also involved in the pathogenesis of human type 1 diabetes, it remains to be investigated, whether supplementation with Zn2+ can at least retard diabetes manifestation in individuals at risk.

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173A.—Do Calcium Supplements Alter Copper and Zinc Status in Postmenopausal Osteoporotic Women? Lowe N.M.,* Jack C.I.A.,[+] Fraser W.D. and Jackson M.J.**** *University of Central Lancashire, Preston U.K. [+]Royal Liverpool and Broad Green University Hospital Trust, Liverpool, U.K. **University of Liverpool, Liverpool, U.K.

Elderly patients with osteoporosis are frequently prescribed Ca supplements to aid in the maintenance of bone mineral density. Cu and Zn also play an important role in bone formation and many elderly subjects have been shown to be marginally deficient in these minerals due to suboptimal dietary intake and reduced absorption. Paradoxically, Ca supplementation may accentuate the problem of poor Zn and Cu status by impairing the absorption of simultaneously ingested Zn and the retention of Cu1,2 . The purpose of this study was to investigate the effect of Ca supplementation on indices of Zn and Cu status, and fractional zinc absorption (FZA) in a group of 11 postmenopausal osteoporotic women. Their dietary mineral intake was determined using a 4-day weighed intake diary. Measurements of plasma Zn concentration (PZn) and plasma Cu concentration (PCu) were made at the start of the study by atomic absorption spectrophotometry. FZA was determined using a dual stable isotope technique in which an oral (2.0 mg67Zn) and intravenous (0.4 mg70Zn) were given simultaneously, following a standard breakfast meal. Plasma isotope enrichment was determined by Inductively Coupled Plasma Mass Spectrometry. Patients were given Ca supplements

Table 1

Subject characteristics (n = 11)

	Median	Range
Age (years)	69	62–77
Years since menopause	17	11–36
Dietary intake of Copper (mg/day)	1.02	0.61–1.47
Zinc (mg/day)	9.30	6.5–13.1
Calcium (mg/day)	969	552–1463

Table 2

Indices of Zn and Cu status (mean (SD), n = 11

	Before Ca supplementation	After Ca supplementation
PZn (ug/ml)	0.63 (0.10)	0.65 (0.07)
Pcu (ug/ml)	1.15 (0.15)	1.13 (0.17)
FZA	0.22 (0.05)	0.24 (0.08)

(Calcichew, Shire Pharmaceuticals, Andover, Hants, UK.), 1000 mg Ca/day as CaCO3 for 28 days and the measurements were repeated.

There was no significant effect of 1000 mg Ca taken daily for 28 days on plasma Zn or Cu concentration or FZA . The long term effects of Ca supplementation on Zn and Cu status require further research.

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2. Snedeker SM, Smith SA & Greger JL (1982) Journal of Nutrition 112, 135–143.

174A.—Low Intracellular Zinc Levels Impairs the Translocation of Activated NFkB to the Nuclei Through Tubulin Depolymerization. Gerardo G. Mackenzie,* M. Paola Zago,* Alejandra G. Erlejman,* Carl L. Keen and Patricia I. Oteiza.*** *IQUIFIB-Department of Biological Chemistry (CONICET-UBA), School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina and **Departments of Nutrition and [+]Internal Medicine, University of California Davis, Davis CA 95616, USA.

NF-B nuclear binding activity was previously found to be markedly lower in rat and cell models of zinc deficiency. In the work herein, we have studied how variations in extracellular zinc levels can modulate the different steps involved in NF-B activation using human neuroblastoma IMR-32 cells. Cells were incubated in control medium or in DTPA-chelated medium containing 1.5, 5, 15 or 50 M zinc. N-6-(6-methoxy-8-quinolyl)-p-toluenesulfonamide-reactive zinc (TSQ-zinc) decreased rapidly (within 3 h) in the cells exposed to 1.5 or 5M zinc. At 24 h, TSQ-zinc was 65 to 70% the amounts in the other groups. Low intracellular zinc levels were associated with the activation of NF-B, based on high levels of IB phophorylation, low IB concentrations and high NF-B binding activity in total cell fractions from cells exposed to 1.5 and 5 M zinc. However, this activation was also associated with the accumulation of the active dimer (RelA-p50) in the cytosol and a low ratio of nuclear/cytosolic NF-B binding activity. The impairment associated with low intracellular zinc, in the translocation of the active NF-B from the cytosol to the nuclei was accompanied by a decreased transactivating activity of an endogenous NF-B-driven gene (Ikba) and of a reporter gene (pNF-B-luc). Tubulin depolymerization has been described to activate NF-B and simultaneously impair the translocation of the active dimer from the cytosol to the nuclei. In IMR-32 cells incubated in media containing low zinc concentrations (1.5 or 5 M), a low rate of in vitro tubulin polymerization was measured compared to the other groups, although the total -tubulin concentration was similar among the groups. We conclude that low intracellular zinc concentrations induces tubulin depolymerization, which may be an important signal for NF-B activation. However, NF-B nuclear translocation is impaired, which inhibits the transactivation of NF-B-driven genes. This could affect cell survival, and be an important factor in zinc-deficiency-associated teratogenicity.

175A.—Observation of the Ratio of Metallothionein Isoforms in Each Fraction After Cell Fractionation of Mouse Liver. Takeshi Minami,* Kanenobu Kubo and Seiji Ichida.[+] *Department of Life Sciences, School of Science and

Engineering, Kinki University, Osaka 577-8502, JAPAN and +School of Pharmaceutical Sciences, Kinki University, Osaka 577-8502, JAPAN.

Study of the function of metallothionein (MT) isoforms has gradually progressed. Capillary zone electrophoresis (CZE) analysis using a polyacrylamide-coated capillary can well separate MT isoforms under a neutral pH without any detergents. CZE can apply nanoliter volume of specimen and separate MT-1 and MT-2 isoforms within 15 min. Mouse liver was removed time-dependently after the subcutaneous injection of zinc (50 mg/kg) and cell fractionation was treated. MT-1 and MT-2 isoforms were detected in nuclear and cytosol fractions between 6 and 24 hours after Zn injection. The ratios of MT-1/MT-2 were significantly difference between cytosol and nuclear fractions at 24 hours. MT-1 was less than MT-2 in nuclear fraction, while MT-1 was much greater than that of MT-2 in cytosol fraction. Furthermore, MT-1 was not detected in cytosol fraction at 48 hours, although MT-2 was detected in cytosol fraction and both isoforms were in nuclear fraction. No MT isoforms were detected in mitochondrial and microsomal fractions during experiments. There are many reports finding MT in cytosol and nucleus by immunohistochemical staining. However, the function of each isoform in nucleus and cytosol is not clear still now. From the present study, the function of each isoform in the fractions may be clear in a near future.

176A.—Induction of Cycling Catch-up Growth by the Idiorrhythmic Dose-rate Feeding with a Non-energetic Nutrient (Zinc). Berislav Momčilovič. Institute for Medical Research and Occupational Health, P.O.B. 291, Zagreb, CROATIA.

Catch-up growth was studied by varying the content of energetic nutrients; proteins, fats, carbohydrates, and their relative proportions. These models are intuitive because it is not possible to fully quantify the intake relative to the duration of the catch-up growth period and the number of body weight cycles (BWC) is arbitrary. Idiorrhythmic dose-rate feeding experimental design offers to quantify the variability in the nutrient intake by spatio–temporal structuring of the diet, such that the average dietary Zn concentration (modulo, M) remains constant across different groups throughout the experiment (epoch, E), i.e., idiorrhythms involve offering the diet with n times the overall Zn concentration (M) only every nth day with Zn-deficient diet offered on the other days (Momčilovič, J.Nutr 1995;125: 2687). In this experiment Adequate (M12) and High (M48) diets provided a daily average of 12 and 48 mg Zn kg-1d-1 over a 48 day E, respectively. Every M has 8 different idiorrhythms (I); I = 36/3 means feeding of the diet containing 36 mg Zn kg-1 every 3rd day with no Zn in the diet on the other 2 days. Body weight cycling plots (Yes/No; statistically Not Significant) of weaning rats were analyzed by Visual examination and by Fourier series.

Modulo	M12 (Adequate Zn)								M48 (High Zn)							
Idiorrhythm	12/1	24/2	36/3	48/4	60/5	72/6	84/7	96/8	48/1	96/2	144/3	192/4	240/5	288/6	336/7	382/8
Visual	N	N	N	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y
Fourier	N	N	N	NS	NS	NS	NS	NS	N	N	N	N	N	N	N	N

Idiorrhythmic dose-rate feeding with a non-energetic nutrient (Zn) is an exact quantitative way to induce BWC; a new tool for the study of the associated health risks, and obesity, and a potential method for in vivo cell synchronization. The Fourier spectral analysis of time series has a detection limit of 300–600 repeated cycles what renders the method unsuitable for the analysis of a small number of cycles in a whole body human and animal experimentation. Visual examination of the plots showed qualitative accuracy which remained to be quantified (see further: Momčilovič, B: Menu Beyond the RDA's etc, TEMA11 this Symposium).

177A.—Menu Beyond the Recommended Dietary Allowances (RDAs) – Idiorrhythmic Zinc Dose-rate Feeding Proves that Adequate Zinc Nutrition is Possible when Zinc is Missed from the Diet for Five Days. Berislav Momčilovič. Institute for Medical Research and Occupational Health, P.O.B. 291, Zagreb, CROATIA.

The daily based RDA's elegantly avoid the fundamental issue on how an intake exceeding requirements could compensate for a previously insufficient one. Only recently, the attempts to provide a rational trace element (TE) supplementation in many parts of the world gave a new dimension to the old issue. Zn was administered according to the idiorrhythmic dose-rate feeding model; the overall amount of available Zn (Modulo, M) is partitioned so that the Zn dosing days (Zn DD) are separated by a no-Zn DD throughout the experiment (Epoch, E) (Momčilovič, J. Nutr, 1995;125:2687). The effect of three different M (M3, M12, and M48; i.e., 3, 12, and 48 mg Zn kg-1 d-1) every M with 8 idiorrhythms (I = M/1–8M/8) on body weight gain (BWG) was studied. To compensate for body weight cycling (BWC), standard results (S) on BWG were linearized (L); the tortuous line was transformed such that every increment and decrement was summed up.

All M3 I's showed significant cycling; M12 and M48 I's started cycling from I = 48/4 and I = 288/6, respectively. Apparently, the BWC reflects a subtle dietary imbalances which may result from a non-linear increase of the metabolic turnover. Thus, I = 36/3 and I = 240/5 fully compensated for 2 and 5 days without Zn, respectively. Evidently, few days without a non-energetic nutrient (Zn) from the diet can be fully compensated. This

Modulo (mg Znkg-1 d-1)		Idiorrhythm (dnthMx/dnth mg Zn kg-1 dnth-1; dnth Zn dosing day) [a,b $p < 0.05$ for the same M]							
		M/1	2M/2	3M/3	4M/4	5M/5	6M/6	7M/7	8M/8
M3 Low Zn	S	124.6a	125.3a	91.4a	112.8a	151.3a	1o4.7a	88.3a	100.3a
	L	244.5b	224.3b	224.3b	275.6b	345.6b	271.9b	263.3b	286.2b
M12 Adequate Zn	S	245.5a	248.6a	269.4a	181.7a	208.4a	202.6a	189.3a	169.7a
	L	247.1a	251.2a	269.8a	318.1b	219.5b	256.9b	271.8b	254.8b
M48 High Zn	S	366.5a	362.4a	357.3a	378.9a	341.7a	332.4a	322.9a	253.6a
	L	374.6a	367.5a	364.1a	393.4a	364.1a	353.0b	372.5b	349.0b

finding opens a new venue on how to structure an adequate diet beyond the RDA's.

178A.—Zinc and Copper Levels in Esophageal Cancer. A.P.S. Narang. Department of Biochemistry, Dayanand Medical College & Hospital, Ludhiana-141001, Panjab, India.

Esophageal cancer is commonly encountered in Kashmir Valley, upper northern region of India. Copper is biologically essential for normal development, growth and physiological functions of body, but little is known about its clinical significance and prognostic value in cancer. Copper utencils are extensively used by the general population of Kashmir. Copper and zinc levels were estimated in milk, diet and in blood samples of the inhabitants and in patients of esophageal cancer. Significantly higher levels of copper were found in human as compared to cow's milk. Low levels detected for zinc. Similarly, higher levels of copper and decreased values for zinc were found in their daily diet. Increased levels of serum copper and decreased levels of serum zinc were detected in patients of esophageal cancer when compared to normal population.

179A.—Studies on the Relation of Zinc and Thymulin. Qu Ning, Chen jing and Xia Yiming. Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing 100050,China.

Thymulin is a nonapeptide produced by thymic epithelium originally isolated from serum. Its former name is "factor thymic serum". Thymulin requires zinc for its biological activity. To investigate the relationship between zinc and thymulin, Wistar rats were fed with zinc-deficient diet (4 mg/kg) and zinc-supplement diet (125 mg/kg) for 8wks respectively. The concentration of serum zinc was measured by AAS. The thymulin level in serum was determination by HPCE and the thymulin activity was determined by rosette. The results show that the thymulin level and its activity in zinc-deficiency group was lower than that in zinc-supplement group ($p < 0.01$), but there was no difference in serum zinc level between the two groups. These results suggest that zinc deficiency affects the level of thymulin. Further study is needed to investigate if thymulin can be used as an index to assay early zinc deficiency.

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180A.—A Prospective Model for Human Zinc Homeostasis. Donald Oberleas. Texas Tech University Emeritus, Lubbock, Texas.

A physiological mechanism for zinc homeostasis has been developed with a rat model (Oberleas, D, J. Inorg. Biochem. 62: 231–241, 1996). The model emphasizes the importance of the pancreas, the relationship of dietary phytate in altering zinc homeostasis and has been confirmed (Oberleas, D & Kwun, I, Metal Ions in Biol. Med.-5, pp. 140–145, 1998). Qualitatively the zinc homeostasis mechanism is identical for all species from rainbow trout to humans. This report thoroughly reviews the literature and creates a human model for zinc homeostasis from published data. Computer models have been developed for humans that reflect the distribution and redistribution of zinc (Wastney, ME & Henkin, RI, Prog. Food Nutr., Sci. 12: 243–254, 1988; Lowe, NM et al., Am. J. Clin. Nutr. 65: 1810–1819, 1997). No computer model reflects the importance of the pancreas in zinc homeostasis nor the flux of zinc secreted by the pancreas. Daily zinc intake in adult humans has been estimated at 9 to 12 mg/day by many investigators (Osis, D et al., Am. J. Clin. Nutr. 25: 582–588, 1972; Kelsay, JL et al., Am. J. Clin. Nutr. 32: 2307–2311, 1979; Hunt, IF et al., Am. J. Clin. Nutr. 32: 1511–1518, 1979). The absolute replacement needs for zinc have been estimated as 5 mg/d (Kirchgessner, M, TEMA-8:4–21, 1993). Flow rate of pancreatic fluid has been estimated by several investigators and is variable throughout the day. Cannulation of the common bile duct of humans is difficult though duodenoscopes are available. Some studies used duodenal aspiration and are not reliable. Estimates of pancreatic fluid flow using pancreatic fistulas range from 801 to 1,770 mL/24 h with one observation of 3,300 mL/24 h (Miller, JM & Wiper, TB, Ann. Surg. 120: 852–872, 1944: Sinclair, ISR, Br. J. Surg. 44: 250–262,1956; Elmslie, RG et al., Ann. Surg. 160: 937–949, 1964). Neither correction for occlusion by the fistula tube nor dimensions of the tube were recorded. Patients were fasted in most studies and fasting affects pancreatic fluid flow rate (Sinclair, ISR, Br. J. Surg. 44: 250–262, 1956). Zinc analyses of 0–2.32 mg/L were reported for duodenal aspirates (Sullivan, JF et al., Gastroenterol. 48: 438–443, 1965). Conservative estimates for human pancreatic zinc secretion are 20 to 40 mg/d.

That represents 1 ö 2% of total body zinc. Except for an accurate concentration of zinc in pancreatic fluid, sufficient data are available to establish a model for human zinc homeostasis.

181A.—Zinc Deprivation by DTPA Impairs the Calcium Second Messenger in 3T3 Fibroblasts. O'Dell, B.L., MacDonald, R.S. and Browning, J.D. Departments of Biochemistry and Nutritional Sciences, University of Missouri, Columbia, USA.

Zinc deficiency in animals results in impaired calcium uptake by platelets when they are stimulated by aggregating agents and by brain synaptic vesicles when they are stimulated by the neurotransmitter, glutamate. This study extends these observations of apparent impaired of plasma membrane function in tissues of zinc-deprived animals to zinc-deprived Swiss 3T3 fibroblasts. The cells were depleted of growth factors (GFs) by growing for 2 d in culture medium (DMEM) containing 2% calf serum. They were then grown in serum-free DMEM containing 0.6 mM diethylenetriaminepentaacetate (DTPA), an impermeant chelator, and stimulated by a single GF, platelet derived growth factor (PDGF) or insulin-like growth factor-I (IGF-I), with or without addition of 0.4 mM zinc. After stimulation with a GF and Zn for 2 h in a factorial design, 45Ca uptake was measured during a period of 1 min. Cells stimulated with PDGF or IGF-I in the presence of added zinc took up calcium at approximately 2-fold the rate of those without added zinc. Calcium in the culture medium and its cellular influx upon GF stimulation are essential for 3T3 cell proliferation. Proliferation, as measured by thymidine incorporation into DNA, was decreased also by lack of bioavailable zinc. The maximal effect of zinc deprivation on thymidine incorporation occurs 12–14 h after IGF-I stimulation whereas the maximal effect on Ca uptake occurs within 1–2 h. Thus, it appears that failure of cell proliferation and decreased DNA synthesis, which occurs in zinc deficiency, results from failure of a calcium second messenger, i.e., zinc deprivation results in decreased Ca uptake from the medium. It is postulated that a key role of zinc is to shelter the function of a calcium channel protein that is essential for transduction of growth factor signals. Zinc's first limiting role appears to be protective rather than catalytic.

182A.—Serum Calcium, Zinc, and Copper in Relation to Biomarkers of Lead and Cadmium in Men. Alica Pizent, Jasna Jurasović and Spomenka Telišman. Institute for Medical Research and Occupational Health, Zagreb, Croatia.

Many experimental animal studies have shown that relatively high doses of calcium (Ca) and zinc (Zn), and possibly copper (Cu), can reduce the toxicity of several metals including lead (Pb) and cadmium (Cd). On the other hand, very few data are available on the possible influence of exposure to Pb and/or Cd on the Ca, Zn, and Cu status in humans. The present study evaluates the combined influence of Pb, Cd, age, smoking habits, and alcohol consumption on serum concentrations of calcium (SCa), zinc (SZn), and copper (SCu) in male subjects with chronic low to moderate Pb exposure. In a cross-sectional study of 299 healthy male Croatian subjects 20–55 years of age, including 143 Pb workers and 156 control subjects, the interrelationship of blood lead (BPb), activity of d-aminolevulinic acid dehydratase (ALAD), erythrocyte protoporphyrin (EP), blood cadmium (BCd), age, smoking habits, and alcohol consumption with respect to SCa, SZn, and SCu levels was calculated by forward stepwise multiple regression. In separate regression models considering each of the biomarkers of Pb (BPb, ALAD, and EP) with the remaining potential explanatory variables (BCd, age, smoking, and alcohol), a decrease in SCa was significantly predictive by an increasing EP ($P < 10–5$), or by an increasing log BPb ($P < 0.0001$), or by a decreasing ALAD ($P < 0.001$). A decrease in SZn was significantly predictive by a decreasing ALAD ($P < 10–13$), or by an increasing log BPb ($P < 10–13$), or by an increasing EP ($P < 0.005$) and BCd ($P < 0.05$). A decrease in SCu was significantly predictive by an increasing log BPb ($P < 0.0005$) and a decreasing smoking ($P < 0.005$) and age ($P < 0.05$), or by a decreasing ALAD ($P < 0.01$), smoking ($P < 0.01$), and age ($P < 0.05$). The study results indicate that chronic moderate Pb exposure can significantly decrease SZn and SCa, and to a lesser extent SCu, whereas Cd exposure may contribute to a decrease in SZn. This is relevant when evaluating possible mechanisms of the Pb- and/or Cd-induced adverse health effects in humans.

183A.—Concentrations of Zinc and Hexa- and Pentaphosphate Fractions in Brazilian Diets with Equilibrated Macronutrient Contents. Marisilda de A. Ribeiro,* Débora I.T. Fávaro,[+] Ralf Greiner,[#] Maria Izabel de O. Eiras,* Kátia M. Baba[†] and Silvia M.F. Cozzolino.[†] *Universidade Federal de Pernambuco, Recife, Pe-Brasil, [+]Instituto de Pesquisas Energéticas e Nucleares, São Paulo, SP-Brasil; [#]Federal Research Centre For Nutrition, Karlsruhe, Germany, [†]Universidade de São Paulo, São Paulo-Brasil.

Studies on zinc reveal a strong relation between dietary deficiencies and metabolic disturbances. Under this aspect, the diet depends on a quantitative and qualitative composition of macronutrients, on the type of available proteins, on the zinc concentration, and on phytate in it. A control of the negative effect of phytates is necessary in countries, where cereals and leguminoses represent the predominant part of the daily diet. The determination of specific inositol phosphate fractions, which may decrease the bioavailability of zinc, becomes possible applying new analytical techniques. Concentrations of zinc and of P5 and P6 inositol fractions were determined in a controlled diet over a period of seven days. The diet included four meals (breakfast, lunch, snack and dinner) and was equilibrated in macronutrients. The meals were consumed by 12 volunteers and in accordance with the energy necessity of each one (RDA).1 The centesimal composition of the diets was analyzed by direct methods recommended by the AOAC. The percentage of proteins ranged between 15–17%, lipids between 23–27% and carbohydrate between 52–60% in relation to the total energy value (calories). The determination of Zn and of P5 and P6 fractions was carried out by applying neutron activation analysis (NAA) 2 and HPLC 3 respectively. The observed zinc concentrations varied between 10,71 −15,88 mg/day, and were herewith above the recommended dietary allowance (RDA) values. 4 The fractions of P6 e P5 in the diets was not detectable (<0,01). Conclusion: 1) an equilibrated brazilian diet is sufficient to cover the recommended values of zinc, 2) the presence of phytate in diets

containing rice and beans seems to be without influence on the bioavailability of zinc.

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184A.—Comparison of Organic vs. Inorganic Sources of Zinc Supplementation on Zinc Retention in Young Pigs. Michael J. Rincker, Gretchen M. Hill, Jane E. Link, Jason E. Rowntree, Dana M. Dvoracek-Driksna and Jessica G. Green. Michigan State University, East Lansing, MI, USA.

Numerous studies have compared organic and inorganic sources of Zn; however, results have varied. Diets did not contain solely organic or inorganic Zn and often an additional Zn supplement was supplied in the mineral premix. A beneficial response in both humans and animals to pharmacological supplementation of Zn has been reported (Sazawal et al., 1995). Thus, the objective of this experiment was to compare Zn Oxide (ZnO) with Zn Methionine (ZnM) supplementation in young pigs. Twenty-four barrows (18 ± 2 d; 6.48 kg) were randomly allotted by body weight and litter and fed (14 d) one of three dietary treatments formulated to meet NRC recommendations (1998) (1) negative control (NC), no supplemental zinc source; (2) NC + 2,000 ppm ZnO; and (3) NC + 2,000 ppm ZnM. Pigs were individually housed in metabolism pens and feces and urine were collected daily (24 hr period). Blood was collected on d 0, 7, and 14. Pigs were euthanized on d 14, and liver and kidney samples were collected. Feces, urine, plasma, and tissue were analyzed for Cu, Fe, and Zn concentrations by atomic absorption spectrometry. Plasma and hepatic Cu and Fe concentrations did not differ. Although kidney Cu was increased ($P < 0.05$) in ZnO and ZnM compared with NC (18.9 and 21.0 vs 6.4 ug/g, respectively), renal Fe concentration did not differ due to pharmacological Zn treatments. Total fecal and urinary Cu excretion did not differ between treatments; however, total Cu retention was increased ($P < 0.001$) in NC and ZnO compared with ZnM (0.8 and 0.7 vs 0.3 mg, respectively). Even though total fecal and urinary Fe excretion did not differ between treatments, total Fe retention was greater ($P < 0.05$) in ZnO than ZnM (50.1 vs 33.8 mg, respectively) and both were greater than NC (27.2 mg). Plasma Zn concentration was higher ($P < 0.0001$) in ZnO and ZnM compared with NC (1.1 and 1.2 vs 0.5 mg/L). Hepatic Zn concentrations followed the same pattern (341.8 and 373.2 vs 52.6 ug/g, $P < 0.0003$); however, renal Zn was greater ($P < 0.005$) in NC than in ZnO and ZnM (16.6 vs 8.6 and 8.8 ug/g, respectively). Animals fed pharmacological Zn had similar Zn retention (139.5 vs 122.1 mg) and fecal excretion (172 vs 256 mg); however, those fed ZnM had greater urinary excretion (6.4 vs 2.0 mg, $P < 0.003$) compared with pigs fed ZnO. Therefore, ZnM appears to result in increased Zn absorption and in a different metabolic management to achieve homeostasis.

185A.—Zinc Treatment, Metallothionein and Carbohydrate Metabolism in Early Endotoxaemia. Allan M. Rofe, Jeffrey C. Philcox and Peter Coyle. Division of Clinical Biochemistry, Institute of Medical and Veterinary Science, Adelaide 5000 Australia.

The advantage to the host of the hypozincaemia and hepatic zinc (Zn) accumulation seen during inflammation/infection is still unclear. The mechanisms underlying these changes in Zn homeostasis are established and are directly linked to the rapid increase in hepatic metallothionein (MT) following the initial inflammatory/infectious insult. Investigation of metabolism in normal (MT+/+) and MT I&II knockout (MT−/−) mice at 16–48 h after endotoxin (LPS) injection (1,2) have shown a decrease in the ability of MT−/− mice to maintain hepatic glucose homeostasis, coinciding with the lack of hepatic Zn accumulation. This study examined the metabolic effects of manipulating the plasma Zn (pZn), hepatic Zn (hZn) and hepatic MT (hMT) concentrations during the first 6h following LPS injection. Fed, normal,(MT+/+ and MT−/− mice were injected with either LPS (1 ug/g body wt, ip); Zn (2 ug/g body wt as ZnSO4 sc) or Zn + LPS, and killed at time points over 6h, food being withheld. Raising pZn by 4–5 fold not alter the initial metabolic response to LPS (decreased liver and blood glucose concentrations, glycogen depletion) in either genotype, even though the hZn was increased by up to 38% in MT+/+ due to the increase in MT . Therefore, under these conditions, pZn concentration, or additional hZn or hMT did not change key metabolic indices. Splenomegaly was observed in only the LPS injected MT+/+ mice and this was partially prevented by Zn treatment. In addition, Zn treatment alone, which increased hZn by 27% in MT+/+ mice, significantly decreased the glycogen depletion seen with fasting alone in the MT+/+ but not MT−/− mice. Additional investigations into glycogen metabolism during meal-feeding revealed an association between decreased hepatic ATP levels and decreased glycogen accumulation in MT−/− mice which further implicates MT or Zn-MT in the regulation of hepatic metabolism.

186A.—Zinc in Colostrum From Two Groups of Lactating Women. Patricia Ronayne de Ferrer,* Adriana Weisstaub,* María L. Portela,* Nora López, José Ceriani Cernadas,** Laura López,[+] María J. Cuetos[+] and Carlos Ortega Soler.**[+] *Sch. of Pharmacy & Biochemistry, Univ. of Buenos Aires, **Italian Hospital and [+]"D. Paroissien" Hospital, Argentina.

It is well known that zinc (Zn) is an essential element, very important for early growth and development, and immune competence, which deficiency might be especially critical in early life. Recently, it has been suggested that zinc may be a first limiting nutrient in human milk. On the other hand, it has been reported that milk zinc concentrations may vary with geographical area and may differ in groups according to their socio-economic levels. On this basis, we decided to assess Zn levels in colostrum from two groups of lactating women belonging to different socio-economic levels (low income LI and middle-class MC). Samples from 51 LI mothers and 55 MC mothers were collected and kept at -20°C until analyzed by atomic absorption spectrophotometry, prior wet ashing with nitric acid. Zn values (mg/ml) (X+SD) and ranges were the following:

Day	2	3	4	5
LI	9.8+4.1 (1.7–17.2)	9.3+3.5 (4.2–16.5)	8.5+3.2 (3.5–11.8)	8.0+2.7 (5.3–13.6)
MC	8.2+4.4 (1.6–15.8)	9.2+3.2 (3.0–16.3)	7.9+2.8 (3.1–14.4)	10.0+4.3 (4.8–18.2)

Birth weight of LI and MC neonates did not differ (3420 ± 569g vs. 3548 ± 448g, respectively). Colostrum Zn concentrations were similar in both populations. Variation along time was not significant (ANOVA). Zn levels were within normal values, according to published data. Even though there is little information about Zn dietary levels in Argentina, previous studies from our group have shown low Zn intakes in lactating women. This is not reflected in colostrum levels, which agrees with data suggesting that milk Zn amounts are independent of diet. (Research Project B062, University of Buenos Aires).

187A.—Endogenous Loss and Absorption of Zinc From Diets and Supplementation in Persistent Diarrhoea Measured by Stable Isotopes. S.K. Roy,* S.F. Tait,+ T. Fox, K.E. Islam* and A.M. Tomkins.** *Centre for Health and Population Research, Bangladesh (ICDDR,B) GPO Box 128, Dhaka 1000, Bangladesh, and +Institute of Food Research, Norwich, UK. And **Centre for International Child Health, 30, Guilford street London, UK.

Objective: To measure the efficiency of zinc absorption from two different therapeutic diets and medicinal zinc supplementation during and after persistent diarrhea. Methodology: A total of 45 male moderately malnourished children aged 6 to 24 months having persistent diarrhea were studied. Children were randomly allocated into 3 groups. (a) Rice based diet + Zn (b) Chicken diet+Zn (c) Rice based diet+placebo. Children of 2 intervention groups received 20mg elemental zinc/day for 2 weeks. Each group was studied for metabolic balance study for a period of 7 days. Zinc absorption was measured from diet and supplementation using zn70 and zn67 isotope respectively. Endogenous zn, cu and mg loss in stool during and after diarrhea was also quantitated. Results: The average duration of clinical recovery was 5.4 days. The results showed that there was no significant difference between absorption of supplemental zinc from rice based (46.8%) and chicken based (48%) diet during acute stage of diarrhea. Zinc absorption from therapeutic diet was even better whether the source was rice suji (50.4%) or comminuted chicken (48.2%). Though the absorption of both therapeutic and dietary zinc improved slightly with recovery but this difference was not significant. Endogenous zinc loss was significantly higher in acute stage, which reduced at recovery. Conclusion: The results showed that moderately malnourished children suffering from persistent diarrhea had adequate absorption of zinc from either rice or chicken based diets.

188A.—Diurnal and Longitudinal Variations of Zinc Concentration in Human Milk and Maternal Plasma. Erika Sievers,* Hans D. Oldigs+ and Jürgen Schaub. *Municipal Hospital, Department of Pediatrics, Flensburg, +Christian-Albrechts University, Department of Pediatrics, Kiel, Germany.

The zinc (Zn) concentration in human milk declines markedly during the first months of lactation (Acta Paediatr 1992; 81:1–6), diurnal variations in Zn concentration in human milk have been described. This study focuses parallel investigation of Zn in human milk and maternal plasma and to detect variations of the respective concentrations. Eight lactating mothers were investigated during the first 16 ± 1 weeks of lactation in 23 periods of 48 hrs. Parallel collections of milk (fore and hind milk of the side(s) used, n = 513) and blood (n = 92) were done at home at the first breast feeding after 5 a.m. and 6 p.m., respectively. Specimens were stored at–200°C until analysis by atomic absorption spectroscopy. The median Zn concentration declined from 4.4 mg/l at two weeks of lactation to 1.7 mg/l at 16 weeks, whereas no significant decline of maternal plasma concentrations was observed. In the specimens collected in the morning showed higher concentratons of Zn in human milk (9.8%) and plasma (16.7%) compared to evening specimens. These diurnal variations were observed during the entire study period. The initial relation of zinc milk/plasma concentration was >3:1, sharply declining thereafter. These results support the assumption of different reasons for the variations of Zn concentration in human milk and confirm the necessitiy of precise standardisation of investigations on Zn intake of breast-fed infants.

189A.—Urinary Zinc Excretion in Preterm Infants–Influenced by Theophylline Medication? Erika Sievers* and Jürgen Schaub. *Christian-Albrechts University Department of Pediatrics, Kiel, Germany.

The urinary zinc (Zn) concentration in term infants may be influenced by nutrition, age or contamination. Preliminary results were suggestive of an additional effect of theophylline medication in preterm infants (1). It therefore was investigated whether the medication of theophylline, clinically indicated for the treatment of apnoea in preterm infants, may influence urinary Zn excretion in these patients. Midstream urinary samples were twice collected in preterm infants under oral nutrition: 1. Theophylline-treated group A (n = 10), median postconceptual age: 34 weeks (medication) and 38 weeks (without theophylline) 2. Untreated group B, (n = 6), median postconceptual age: 34 and 36 weeks; 3. Untreated term infants (group C, n = 9) at a postconceptual age of 38 weeks. Zn analysis was done by atomic absorption spectroscopy. In group A the median urinary Zn excretion declined from 0.53 (0.1–1.24) to 0.2 (0.1–2.92) mg/L ($p > 0.05$), in group B from 0.37 (0.12–0.96) to 0.31 (0.04–4) mg/l ($p > 0.05$), group C rendered values of 1.1 (0.4–3.02) mg/L. The results of group B and C differed significantly, but group A rendered no significant difference compared to the other groups. Two values exceeding 1 mg/L in

group A were assessed in infants with a dosage exceeding 6 mg theophylline/kg body weight. These results show the high variability of urinary Zn excretion even under standardized conditions but do not confirm a significant influence of theophylline medication on urinary Zn excretion in preterm infants.

1. J Trace Elem Electrolyt Health Dis (1990) 4:31–35.

190A.—Zinc Administration in Nepalese Children with Acute Diarrhea–Who Will Benefit Most? Tor A. Strand,* Ram K. Chandyo,*† Rajiv Bahl, Pushpa R. Sharma,† Håkon Gjessing,* Ramesh K. Adhikari,† Nita Bhandari,** Rune J. Ulvik,†† Maharaj K. Bhan** and Halvor Sommerfelt.*** *Centre for International Heath, University of Bergen, Norway; †Department of Child Health, Tribhuvan University, Kathmandu, Nepal; **Department Paediatrics, All India Institute of Medical Sciences, New Delhi, India; ††Institute of Clinical Biochemistry, University of Bergen, Norway.

The therapeutic effect of zinc on acute diarrhea has been well documented. Moreover, routine zinc supplementation to children reduces the incidence and prevalence of lower respiratory tract infections and diarrhea, especially of severe disease. It has been suggested that children with low tissue zinc concentration and with malnutrition benefit more than other. The additional effects in these subgroups have been inconsistent and relatively low in the trials reporting them. We wanted to examine whether the effect of zinc given to Nepalese children with acute diarrhea was different among various sub-groups. We also wanted to assess the clinical, socioeconomic and anthropometric factors that were associated with low plasma zinc at enrollment. A double blind randomized placebo-controlled trial in children six to 35 months of age was undertaken. Children with diarrhea that had lasted for less than 96 hours were randomized to receive placebo (n = 447) or zinc (n = 448) syrup. The syrup was given daily by field workers and day-wise information on episode characteristics and morbidity was recorded every fifth day. The main outcome was time to recovery from diarrhea. Cox proportional hazards models with interaction terms was used to assess the effect of zinc in different sub-groups and to estimate the precision of the interaction. The unadjusted relative hazard (RH) for termination of diarrhea was 1.26 (95% CI: 1.09, 1.46) higher in the zinc than in the placebo group. The effect of zinc was higher in the children who had low plasma zinc levels at enrolment (RH 2.18 versus 1.18, P for the interaction term: 0.01), high fever (RH 2.56 versus 1.22, P for the interaction term: 0.04) or other markers of severe infection. Children that were not breastfed and those that were enrolled during the hot and wet season had also better effect of zinc. Nutritional status did not interact with the effect of zinc. In the linear and logistic regression models, markers of the severity of infection such as body temperature, dehydration and stool frequency were independently and strongly associated with the plasma zinc concentration. Temperature measured at enrolment was negatively and highest correlated with plasma zinc. Age, socioeconomic and nutritional status were to a much smaller degree associated with plasma zinc levels at enrolment.

191A.—Application of Zinquin in a Rat Model of Intestinal Damage and Repair. Cuong D. Tran,* Gordon S. Howarth,+ Jeffrey C. Philcox, Allan M. Rofe,** Peter Zalewski,++ Peter Coyle** and Ross N. Butler.*** *Centre for Paediatric and Adolescent Gastroenterology, Women's and Children's Hospital, North Adelaide, 5006, SA, Australia; +Co-operative Research Centre for Tissue Growth and Repair, North Adelaide, 5006, SA, Australia; **Division of Clinical Biochemistry, IMVS, Adelaide, 5000, SA, Australia; ++Department of Medicine, TQEH, Woodville South, 5011, SA, Australia.

Zinc is protective and enhances epithelial repair in gut diseases. However, little is known about the localisation of endogenous zinc in the gut particularly during the repair phase immediately following cell damage. In this study we investigated the localisation and distribution of zinc in the gut of methotrexate-treated rats. Rats were administered methotrexate (2.5 mg/kg body weight/d) by s.c injections in the suprascapular region on d 1, 2 and 3. Rats were killed at d 0, 5, 8 and 10 after the initial injection and gut tissues were collected for analysis. Zinc localisation and tissue zinc were determined by Zinquin fluorescence and atomic absorption spectrophotometery (AAS), respectively. Maximal damage in the gut occurred at d 5 and repair of the jejunum was virtually completed by d 8. Zinquin fluorescence in untreated rats was greater in the ileum than the jejunal crypts. Methotrexate treatment resulted in diminished fluorescence in the ileum at d 5, which recovered to control levels by d 10, coinciding with an increased in crypt cell numbers. At d 5 no Zinquin fluorescence was evident in the jejunal mucosa of methotrexate-treated animals and by d 8 few jejunal crypts showed some fluorescence compared to controls. The majority of zinc in the gut wall of normal rats was present in the mucosal scrapings, with 94% membrane-bound and 6% cytosolic. The zinc content of the ileum was also 20% higher than from other gut regions determined by AAS in normal rats. Methotrexate administration did not significantly alter gut zinc levels on d 5, 8 and 10, except in the ileum where it was elevated by 62% ($p < 0.05$) on d 5 compared to untreated controls. These findings indicate that increased Zinquin fluorescence parallels the recovery of the mucosal lining after methotrexate and more specifically increased crypt cell numbers during the recovery phase.

192A.—Zinc Intake, Excretion, Balance and Requirement–a Placebo-controlled Double Blind Test in Nonpregnant and Pregnant Women. Jürgen Vormann,* Manfred Anke, Ralf Müller*** and Ullrich Schäfer.***** *Institute of Prevention and Nutrition, Ismaning, Germany, **Institute of Nutrition and Environment, Friedrich Schiller University, Jena, Germany, ***Society of Ecology and Environmental Chemistry Ltd, Erfurt, Germany.

Worldwide, the individual basal requirement and the recommendation for Zn intake by adults differ considerably and have led to a confusing variety of data for both parameters. The effect of 10 mg Zn/day on its absorption, excretion, balance and status was systematically investigated in two double blind tests involving nonpregnant and pregnant women. All of them collected the consumed foods, beverages, sweets and their excretions every day. After dry ashing of the samples at 450 °C Zn was determined by means of ICP–OES. The supplementation of 10 mg Zn/day to a daily intake of 7 mg Zn/day increased the faecal Zn excretion to the same extent in nonpregnant and

pregnant women. The individual basal Zn requirement of the women was covered with the natural Zn intake. Though the Zn balance improved, it remained within the range of ± 10%, which is considered a normal variation range. The Zn content in the whole blood, blood serum and hair did not react to the Zn offer. The SOD concentration of blood, a good indicator of a Zn deficiency, was normal in both groups. The individual basal Zn requirement of nonpregnant and pregnant women with a mixed diet and a medium bioavailability of the rations is <7 mg/day. The recommended Zn intake of nonpregnant and pregnant women with a mixed diet amounts to <10 mg/day.

193A.—The Effect of Marginal Zinc Intake on Measures of Cellular Membrane Function in Men. L.R. Woodhouse,* E. Bonnel,* B. Sutherland[+] and J.C. King.* *USDA/WHNRC, University of California, Davis, and [+]EFNEP, Department of Nutrition, University of California, Davis, Davis, CA 95616, USA.

Zinc (Zn) homeostasis is well regulated, although a chronic marginal intake of Zn may exhaust homeostatic control, resulting in the initial clinical signs of Zn deficiency. The effects of a marginal Zn intake on cellular membrane function were studied in 8 men during a 20-wk metabolic study (5-wk baseline; 10-wk low Zn; 5-wk repletion). Dietary Zn was 4.6 mg/d from a natural foods diet throughout the entire study. This level was based on the Zn recommendations established by the WHO of 4 to 6 mg Zn/d in a diet with high Zn bioavailability. During the baseline and repletion periods, the diet was supplemented with 9.1 mg Zn. Plasma and erythrocyte Zn levels did not change during the entire study. Erythrocyte metallothionein decreased during depletion, but the drop was not significant. Erythrocytes were used as a model to test functional measures of membrane integrity. Erythrocyte osmotic fragility and in vitro Zn uptake increased, but not until the repletion period. Erythrocyte membranes had decreased Zn content, with levels decreasing during depletion and continuing to drop during repletion. Erythrocyte membranes also had decreased activity of alkaline phosphatase during depletion. Erythrocyte membrane thiol residues and activities of thiol-dependent ion pumps (ATPase activities) did not change significantly. The delay in occurrence and lack of return to baseline levels for some of these measures may have been due to a negative effect on erythropoiesis. CBC (complete blood count) data lend support to the hypothesis that erythropoiesis was affected. MCH (mean corpuscular hemoglobin) and RDW (red cell distribution width) were lowest at the end of repletion. MCV (mean corpuscular volume) was highest at the end of repletion. These CBC changes suggest a circulation of older erythrocytes with fewer new erythrocytes produced, and support results of the functional measures of erythrocyte membrane integrity. Erythrocyte sodium efflux increased during depletion, and remained high during repletion, and plasma potassium levels decreased during depletion, suggesting negative effects on cellular membrane ion transport occurring only several weeks after consumption of a marginal Zn intake. It appears that sub-clinical changes are occurring at the membrane level due to the short-term marginal Zn intake, and ion transport may have been affected as well as erythropoiesis. These initial biochemical changes are occurring prior to any overt clinical signs of Zn deficiency, and emphasize the importance of Zn for membrane structure, function, and stability.

194A.—Effects of Modulating the Labile Intracellular Pool of Zinc on HCC2218 Breast Cancer Cell Growth. Bella Wu and Zhaoming Xu. Food, Nutrition and Health Group, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

The labile intracellular pool of zinc has been shown to play a critical role in cell proliferation and the induction of apoptosis. Our aim was to investigate the effects of modulating the labile intracellular pool of zinc on HCC2218 human breast cancer cell growth. HCC2218 breast cancer cells were cultured in RPMI 1640 medium (10% FBS). To deplete labile intracellular pool of zinc, cells were treated with NNN'N'-tetrakis-(2-pyridylmethyl)ethlenediamine (TPEN; 0, 1 or 5 μM). To increase labile intracellular pool of zinc, cells were treated with zinc ionophore sodium pyrithione (0, 0.5, 1, 1.5 or 2 μM) with a supplementation of 20 μM zinc. Modulation of labile intracellular pool of zinc was further achieved by a combination of 10 μM TPEN and 0.5 or 1mM pyrithione. Cells were treated for 1, 2, 6, or 24h. TPEN treatment resulted in a dose-dependent reduction in cell growth (15–67%) regardless of treatment duration, but an increased cytotoxicity was only observed after 5 μM TPEN after 24h treatment. Pyrithione treatment for 1 or 2h essentially had no effect on cell growth. After 6h, high concentration of pyrithione (1–2 μM) reduced cell growth (33–45%). By 24h, very few cells survived pyrithione treatment at all concentrations tested. When the cells were treated with TPEN and pyrithione, 1 μM, but not 0.5 μM, pyrithione increased cell growth by 13–36% and reduced cytotoxicity by 12%. Overall, the results showed that both depletion of the labile intracellular pool of zinc and increase of otherwise adequate labile intracellular pool of zinc resulted in a reduced growth of HCC2218 breast cancer cells, suggesting that an optimal level of the labile intracellular pool of zinc is critical to cell growth.

195A.—Effects of Dietary Zinc Deficiency on N-methyl-N-nitrosourea-induced Mammary Tumorigenesis in Rats. Zhaoming Xu, Samantha Lee and Joseph Leichter. Food, Nutrition and Health Group, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

Zinc has been suggested to play a role in mammary tumorigenesis. The aim of this study was to investigate the effects of dietary zinc intake on N-methyl-N-nitrosourea (MNU)-induced mammary tumorigenesis in sexually matured rats. Female SD rats (21-day-old) were fed on Z3 (3mg zinc/kg diet), Z12 (12mg zinc/kg diet), Z31 (31mg zinc/kg diet), or Z155 (155mg zinc/kg diet) diet ad libitum or pair-fed to the Z3 rats on Z12 (PZ12), Z31 (PZ31) or Z155 (PZ155) diet. On day 50 of age, all rats were injected with MNU (50mg/kg BW, i.p.) to induce mammary tumorigenesis. After 14 weeks, plasma zinc level and feed intake in Z3 rats were significantly reduced, but host body weight gain was unaffected, indicating a marginal zinc deficiency status in Z3 rats. Comparing to Z12, Z31, and Z155 rats, tumor incidence, tumor number, tumor multiplicity, tumor weight, and tumor latency were lower in Z3 rats while tumor burden was unaffected by dietary zinc intake. Comparing to PFZ12, PFZ31, and PFZ155 rats, tumor incidence and tumor number were lower in Z3 rats, but tumor multiplicity, tumor weight, and tumor latency were the same. Tumor number, but not tumor incidence, in PFZ12, PFZ31, and PFZ155 rats was lower than in their corresponding ad libitum control rats. Overall, the results suggested that

marginal zinc deficiency reduced incidence of MNU-induced mammary tumorigenesis along with less number of tumors in sexually mature rats. However, the apparent effect of marginal zinc deficiency on tumor multiplicity, tumor weight, and tumor latency was mainly due to a reduced feed intake associated with zinc deficiency.

196A.—The Method of Capillary Zone Electrophoresis for Determining Thymulin in Serum. Qu Ning, Chen jing and Xia Yiming. Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing 100050, China.

Thymulin is a nonapeptide produced by thymic epithelium originally isolated from serum. Its former name is "factor thymic serum". Thymulin requires zinc for its biological activity.

The determination of Thymulin in serum by capillary zone electrophoresis was described, The optimum ionic concentration and pH of buffer system were examined through orthogonal analysis. The optimum conditions were found to be 0.05 mol/L sodium tetraborate, 0.01 mol/L phosphate buffer at pH 8.9, working voltage at 12 kV and wavelength at 200 nm., The values of relative standard deviation (RSD), recovery and detection limit were 7.62%, 80.28% and 40 ng/ml, respectively. The development of this method provides a new technique for study of the relation of zinc and thymulin.

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197A.—Zinc Deficiency Induces DNA Damage and Compromises DNA Repair Signal Pathways in C6 Glioma Cells. Emily Ho and Bruce N. Ames. University of California, Berkeley, CA and Children's Hospital Oakland Research Institute, (CHORI), Oakland, CA.

18% of the US population consumes less than half the recommended level for zinc. Insufficient zinc intake can impair antioxidant defenses and compromise DNA repair mechanisms, making the cell highly susceptible to oxidative DNA damage. The brain is highly sensitive to zinc deficiency. The goal of this study was to investigate the effect of zinc deficiency on DNA damage, expression of DNA repair enzymes, and downstream signaling events in a cell culture model. C6 glioma cells were fed zinc-free media over a 5-day period. Zinc deficiency inhibited cell growth of C6 cells and increased the fluorescence of dichlorofluroscein (DCF), an oxidant-sensitive fluorescent probe, suggesting that zinc deficiency induced an oxidative stress. Comet assays showed an increase in single strand breaks with zinc deficiency. Zinc deficient C6 cells also exhibited an increase in the expression of the DNA repair enzyme AP endonuclease (APE). Repletion with zinc restored cell growth and reversed DNA damage. APE (which is also known as ref-1) is a multifunctional protein that not only repairs DNA, but controls DNA binding activity, via redox mechanisms, of many transcription factors that may be involved in cancer progression. Electromobility shift assays (EMSA) were performed to assess the ability of p53, NFkappaB and AP1 to bind to consensus DNA sequences. DNA binding activity of these transcription factors were compromised with zinc deficiency. This data demonstrates that zinc deficiency causes DNA damage and induces DNA repair protein expression, but important downstream signals leading to proper DNA repair are lost without zinc. Consequently, zinc deficiency not only causes oxidative stress and induces DNA damage, but also compromises the cell's ability to repair this damage. These observations give a mechanistic link between zinc deficiency and how oxidative stress can signal downstream events leading to a loss of DNA integrity and increase the likelihood of developing cancer. This work strongly suggests that zinc deficiency has a detrimental effect on DNA integrity and emphasizes the importance of good nutrition in the prevention of cancer.

197B.—Nutritional and Genetic Copper Overload in a Mouse Fibroblast Cell Line. Angela D. Armendariz and Christopher D. Vulpe. Department of Nutritional Science and Toxicology, University of California, Berkeley, Berkeley, CA, USA.

Copper is a trace metal required by all organisms for survival. Its capacity to convert between oxidation states accounts for its essential role in many proteins in oxidative metabolism, yet can lead to the generation of free radicals. These conflicting properties demand close regulation of copper levels. Complementary nutritional and genetic models of copper exposure make it an ideal system to evaluate the utility of cDNA microarrays for exposure monitoring. cDNA microarrays are a novel methodology to assess differences in the relative gene expression between two mRNA samples of a large number of genes simultaneously. Multiple studies validate this technology for assessing relative gene expression between two different states including different tissue types, environmental stimuli, health and disease and for following sequential changes in expression at regular intervals after an environmental stimulus. The expression changes provide a global genomic view of the metabolic changes that occur during adaptation to a changing environment. We are evaluating in a mouse fibroblast cell line the utility of cDNA microarrays for monitoring exposure to the essential, yet toxic metal copper. We have induced copper overload in the fibroblasts by the addition of copper-histidine. Fibroblasts from a genetic model of copper overload, Mo brindled, were also used. These cells, copper-treated cells, as well as untreated control cells, were collected after 24 hours, and the RNA was prepared. Copper overload states were monitored by determination of copper concentration using atomic absorption spectrometry. We have investigated changes in gene expression of 10,000 different genes during nutritional copper overload. We are comparing the gene expression patterns in nutritional copper overload to those seen in genetic copper overload.

198B.—Short Term Consumption of a High Sucrose Diet Has a Pro-oxidant Effect and Negatively Affects Copper Status in Rats. J. Busserolles, W. Zimowska, E. Rock, Y. Rayssiguier and A. Mazur. Centre de Recherche en Nutrition Humaine d'Auvergne, Unité des Maladies Métaboliques et Micronutrinents, INRA, Theix, 63122 Saint-Genès-Champanelle, France.

Copper deficiency leads to important alterations in antioxidant defenses. The underlying mechanisms for the detrimental consequences of a high sucrose diet in experimental copper deficiency are not clear. However, the possibility exists that fructose feeding negatively affects copper metabolism and facilitates oxidative damage. These findings led us to study the effect of high sucrose diet, copper adequate, on oxidative stress parameters and copper status.

Weaning male Wistar rats, 3 weeks old, weighing 61 ± 2 g (mean ± SEM) were randomly divided into starch or sucrose groups (8 per group). Diet and distilled water were provided ad libitum for two weeks. The synthetic diets contained (in g/kg): 200 casein, 650 starch or sucrose, 50 corn oil, 50 alphacel, 3 DL-methionine, 2 choline bitartrate, and adequate contents of minerals and vitamins.

Higher plasma TBARS and higher urinary excretion TBARS were found in the sucrose group compared to the starch group suggesting increased production of these substances from lipid peroxidation in vivo. Sucrose rats were characterized by high plasma NOx suggesting a higher NO production. The plasma TG raising effect of sucrose was accompanied by lower a-tocopherol plasma levels. Heart from sucrose fed rats are more susceptible to in vitro peroxidation. Oxidative stress parameters and stress related gene expression using cDNA array were investigated in heart. Results indicate higher expression of MT-HSP70 and vimentin, genes associated with the stress response regulation. Moreover, the Cu-Zn-SOD activity was found lower while Cu-Zn-SOD mRNA level was found higher in the sucrose group. Lower copper erythrocyte and tissue concentration were found in rats fed the sucrose group. Whether the decrease in Cu-Zn-SOD activity is related to copper depletion or whether the protein could be damaged by oxidative stress is unknown.

In conclusion, consumption of a high sucrose diet has a pro-oxidant effect in rats. More work have to be done to clarify the role of copper depletion in the pro-oxidant effect of sucrose.

199B.—Effect of Dietary Copper (Cu) on Risk Factors for Colon Cancer in Healthy Men. Cindy D. Davis. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

Introduction: Colon cancer is the second leading cause of cancer mortality in the United States. One possible dietary factor that may increase susceptibility to colon cancer is inadequate dietary Cu. Recent studies have shown that ingestion of a diet low in Cu significantly increased the formation of carcinogen-induced aberrant crypt foci in rats and intestinal tumors in Min mice, a genetic model for human colon cancer. However, little is known about the effect of dietary Cu on colon cancer susceptibility in humans. Methods: Seventeen healthy, non-smoking men aged 21–52 yr completed a 13 wk controlled feeding study to investigate the effects of low and adequate Cu intakes on Cu nutriture and putative risk factors for colon cancer. The basal diet contained 0.45 mg Cu/2500 kcal. After a 1 wk equilibration period in which the subjects were fed the basal diet supplemented with 1.0 mg Cu/d, subjects were randomly assigned to receive either the basal diet or the basal diet supplemented with 2 mg Cu/d for 6 wk. Afterwards, the subjects immediately crossed over to the other level of Cu for the last 6 wk. Volunteers collected their feces during the equilibration period and during the last 2 wk of the two dietary periods for free radical and fecal water analysis. Results: Because the fecal water constituent data did not follow a normal distribution, data were transformed by using the natural log before statistical analysis. Repeated measures ANOVA revealed a significantly ($p < 0.001$) higher free radical production [1.73 (1.56–1.91) vs 1.27 (1.13–1.42) mol methanesulfinic acid/kg wet feces; (geometric means + 1 SEM)] in the feces and significantly ($p < 0.0001$) lower fecal water Cu concentrations [0.13 (0.11–0.15) vs 0.50 (0.45–0.55) g/mL fecal water] when subjects were fed low dietary Cu than when they were fed adequate dietary Cu. There was a significant negative correlation between fecal free radical production and fecal water Cu concentrations ($r = -0.28$, $p < 0.05$, Spearman correlation coefficient). Dietary Cu did not significantly affect fecal water pH, iron or zinc concentrations. Because of the large interindividual variability in alkaline phosphatase acitivity, nonparametric ANOVA was utilized. Subjects had significantly ($p < 0.007$) higher intestinal alkaline phosphatase activity when they were fed low dietary Cu than when they were fed adequate dietary Cu (median values of 1.34 vs 0.52 units/mL, respectively). In contrast to the fecal analysis, hematological indicators of Cu status (serum Cu, ceruloplasmin activity and LDL cholesterol concentrations) were not significantly affected by the dietary treatments. Discussion: These results suggest that low dietary Cu adversely affects fecal free radical production and fecal water alkaline phosphatase activity which are putative risk factors for colon cancer.

200B.—Dietary Copper Alters Basal and Stimulated Lipolysis of Subcutaneous Adipose Tissue in Finishing Steers. K.L. Dorton, L.R. Johnson and T.E. Engle. Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523-1171, USA.

Previous studies have shown that copper (Cu) addition to feedlot diets low in Cu reduced subcutaneous adipose tissue (backfat) deposition in steers (J. Anim. Sci. 75:3057–3065, 1997; J. Anim. Sci. 78:2452–2458, 2000). Therefore, the present study was conducted to investigate the effect of dietary Cu on subcutaneous adipose tissue lipolytic rates in feedlot steers. Forty-eight, individually fed, Angus steers (220 kg ± 9.1) were utilized. Steers were stratified by body weight and initial liver Cu concentration and randomly assigned to one of five treatments. Treatments consisted of: 1) control (no supplemental Cu), 2) 10 mg Cu/kg DM from CuSO4, 3) 10 mg Cu/kg DM from a Cu amino acid complex (Availa Cu), 4) 20 mg Cu/kg DM from CuSO4, and 5) 20 mg Cu/kg DM from Availa Cu. Steers were fed an alfalfa-corn based growing diet for 56 days then switched to a high concentrate finishing diet for 144 days. Prior to slaughter, subcutaneous adipose tissue biopsies were obtained from three steers per treatment to determine basal and norepinephrine-stimulated lipolytic rates in vitro. During the growing and finishing phases, body weights, average daily gains,

and feed efficiencies were similar between control and Cu supplemented steers. On day 56 of the growing phase and day 112 of the finishing phase, Cu supplemented steers had higher ($P < 0.01$) liver Cu concentrations than controls. Steers receiving 20 mg Cu/kg DM had higher ($P < 0.01$) liver Cu concentrations than steers receiving 10 mg Cu/kg DM. Backfat depth tended ($P < 0.12$) to be reduced in steers supplemented with CuSO4 relative to controls. Basal ($P < 0.06$) and norepinephrine-stimulated ($P < 0.04$) lipolytic rates of subcutaneous adipose tissue were higher in Cu supplemented steers. These results suggest that Cu supplementation has minimal effects on performance; however, it appears that Cu may play a role in subcutaneous adipose tissue metabolism.

201B.—Functional Elements in the Menkes Disease Gene Promoter. Edward Harris,* Manchi Reddy* and Sudeep Majumdar.* *Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX.

The promoter for the Menkes disease gene extends more than 2.0 kb from the transcription start site at exon 1. Two domains are discernible. A proximal domain with cis-acting motifs housed with a 98 bp tri-repeat and a distal element composed of nucleotide sequence resembling motifs that recognize gap genes that control development in Drosophila melanogaster. Because ATP7A, a Cu-ATPase gene product, is differentially expressed in development and in select tissues, we have been interested in learning if epigenetic and tissue-specific expression lies with the promoter and DNA-binding proteins that engage distal elements. Cloning and sequencing a 2.2 kb PvuII fragment from human DNA revealed a motif 5′-ACACAAAAAAATA that has sequence similarity to the binding site for the hunchback gene product (Hb) in Drosophila. A putative forkhead motif (HFH-2) was immediately adjacent and partially overlapped the Hb site. Eliminating the distal elements substantially enhanced promoter activity in HepG2 and SY5Y cells, suggesting Hb/HFH-2 suppressed promoter function. Mutagenesis of a C and three adjoining bases in the A-string of Hb, however, surprisingly weakened or abolished promoter activity in three cell lines. SV40 promoter activity was strongly enhanced when a 148 bp fragment with wild-type Hb/HFH-2 sequences was ligated upstream; a fragment with mutated sequences was not stimulatory. Nuclear extracts of SY5Y and HepG2 cells produced distinct gel shift patterns with DNA probes containing the Hb/HFH-2 sites. Thus ATP7A expression appears to be a function of both proximal and distal promoter elements, the latter may coordinate tissue-specific expression of the MNK gene.

202B.—Oxidative Stress Resulting from Inhibition of the Mitochondrial Electron Transport Chain Contributes to the Induction of Hepatic Heme Oxygenase-1 in Copper-deficient Rats. W. Thomas Johnson. United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202-9034.

Studies in rats have demonstrated that copper deficiency increases hepatic heme oxygenase (HO) activity, but it is not clear whether the increase in activity represents an increase in the expression of heme oxygenase or an increase in the expression of NADPH:cytochrome P450 reductase (P450Red) which is required for HO activity. Accordingly, one goal of this study was to determine whether the increase in HO activity caused by copper deficiency reflects an increase in the expression of HO-1, which is an inducible form of HO, or an increase in P450Red. A second goal was to determine if mitochondrial stress contributes to the induction of heme oxygenase by copper deficiency. Weanling rats were fed either Cu-deficient (0.3 mg Cu/kg) or Cu-adequate (5.4 mg Cu/kg) diets for 5 weeks. Optical density units obtained from scanning densitometry (mean ± SEM) were 4.59 ± 0.17 and 2.66 ± 0.34 for HO-1 ($P < 0.05$, one-tail t-test) and 1.11 ± 0.08 and 0.99 ± 0.15 for P450R ($P > 0.05$) in Cu-deficient ($N = 10$) and control rats ($N = 9$), respectively. This finding indicates that Cu deficiency increases HO-1 but has no effect on P450R and that induction of HO-1 is a likely explanation for increased hepatic HO activity caused by Cu deficiency. Activities of mitochondrial NADH:cytochrome c reductase (NADHCytR), succinate:cytochrome c reductase (SucCytR), and cytochrome c oxidase (CCO) were inhibited 70%, 41% and 31% ($P < 0.05$), respectively, in Cu-deficient rats. In addition, HO-1 content in copper-deficient rats was inversely correlated with NADHCytR ($R2 = 0.6$, $P < 0.05$). HO-1 content also exhibited negative exponential associations ($P < 0.001$) with CCO activity and NADHCytR activity across both dietary groups. A mechanism for HO-1 induction during Cu deficiency may involve increased mitochondrial oxidative stress resulting from decreased cytochrome c oxidase and respiratory complex I activities. In the present study, rates of hydrogen peroxide production (mean ± SEM) by isolated hepatic mitochondria in the presence of 10 mM glutamate were 1.25 ± 0.18 and 0.84 ± 0.11 pmol/(sec x mg protein) ($P < 0.05$) for Cu-deficient and control rats, respectively. Hydrogen peroxide is an inducer of HO-1 and thus, the increase in HO-1 during Cu deficiency may be at least a partial consequence of increased cellular concentrations of hydrogen peroxide resulting from impaired mitochondrial electron transport.

203B.—Regulation of Transcuprein/alpha-1-inhibitor-3 by Copper and Iron. Theodros Z. Kidane, Louis S.L. Lo, Nanmei Liu and Maria C. Linder. California Sate University, Fullerton, CA, USA.

Transcuprein, a 270 kDa protein that binds Cu when it enters the blood plasma, was first identified in rats by size exclusion chromatography of plasma taken immediately after injection of tracer 67Cu. Based on amino acid sequence, we identified the major subunit as the macroglobulin, alpha-1-inhibitor-3 (a1I3), and obtained initial evidence that expression is regulated by Cu and Fe. Northern analysis and quantitative PCR of livers from rats, made Cu deficient by placing them on a low Cu diet from weaning, indicated that there was a doubling of mRNA for transcuprein/a1I3; acute treatment with Fe dextran also increased the mRNA. To further explore regulation, we examined the a1I3 promoter and detected several potential metal and Cu regulatory elements. dsDNA oligomers (57–67 nucleotides) incorporating some of these elements were incubated with nuclear extracts of livers from Cu deficient, normal and Fe treated rats, to detect binding in electrophoretic mobility shift assays (EMSA). Specific binding and response to Fe was detected for oligomers with 3 different potential regulatory

elements. Methodology for Real Time PCR of a1I3 mRNA was developed and applied to rat hepatoma cells (H4-II-E-C3) treated with Fe, or with triethylenetetraamine (to deplete Cu), or desferrioxamine (to deplete Fe). No differences in mRNA were detected. However, expression was quite low. Thus, means of enhancing expression, and effects of inhibitors of transcription, are being explored.

204B.—Characterization of Mouse Embryonic Cells Deficient in the Ctr1 High Affinity Copper. Transporter. Jaekwon Lee,* Michael J. Petris[+] and Dennis J. Thiele.* *Department of Biological Chemistry, The University of Michigan Medical School, Ann Arbor, MI and [+]Department of Nutritional Sciences, University of Missouri-Columbia, Columbia, MO.

The trace metal copper is a cofactor for a number of enzymes which have critical roles in biological processes. Consequently, Cu uptake at the cell surface and incorporation of Cu into Cu-requiring proteins are essential events in normal physiology. Ctr1 protein in human and mouse was identified as an important component of cellular Cu transport activity at the plasma membrane. Ctr1 over-expression in the cells stimulates Cu uptake, and deletion of one allele of Ctr1 gene in mice gives tissue-specific defects in Cu accumulation and in the activities of Cu-containing enzymes. Furthermore, mice deficient for both alleles of Ctr1 gene exhibit profound growth and developmental defects and die in utero in mid-gestation. To further investigate the roles for Ctr1 protein in cellular Cu metabolism, we isolated Ctr1-deficient cells from wild type, heterozygous and homozygous knock out embryos obtained from the inter-cross of Ctr1 heterozygous mice. Mouse embryonic cells expressing no Ctr1 exhibit significant defects in Cu uptake and accumulation and in Cu-dependent enzyme activities. However, in vitro growth of the cells defective in Ctr1 is not significantly different compared with wild type embryonic cells isolated form littermate embryos. Interestingly, Ctr1-deficient cells express residual Cu transport activity which is saturable and possesses biochemical features that are distinct from Ctr1-driven Cu transport. These results demonstrate that although Ctr1 is a major Cu transporter which plays essential roles for Cu acquisition and for mammalian embryonic development, another Cu transporter system identified in Ctr1-deficient cells is sufficient for Cu acquisition that is required for proliferation of mouse embryonic cells in vitro.

205B.—Copper in Cerebrospinal Fluid of Children with Acute Bacterial Meningitis. Marcelo Maturana,* Guillermo Venegas,* Ilse Contreras* and Aldo Rodriguez.[+] *Department of Pediatrics, Faculty of Medicine, University of Concepción, Chile and [+]Faculty of Pharmacology, University of Concepción, Chile.

Copper is an essential trace element for aerobic organisms, with important functions like enzymatic cofactor in respiratory chain, antioxidant protection, neurotransmisor's biosynthesis, connective tissue synthesis, iron metabolism and oxidative stress in inflammatory response. With the aim of to study the cerebrospinal fluid (CSF) level of copper in acute bacterial meningitis, samples of CSF from 9 children with acute bacterial meningitis confirmed with cultures and/or latex test, were used for copper determination by atomic absorption spectrophotometry. Samples of CSF were obtained at hospital's admission and 48 hours after antibiotics therapy. For comparative control group, samples of CSF of 5 children with acute viral meningitis or febrile seizures were used. The mean copper level in CSF obtained at admission of the patients was 46.4 micrograms/ml (3-times over the reference value). In the control group the mean CSF copper level was 16.4 micrograms/ml. The CSF copper level at 48 hours of antibiotics therapy was decreased to mean 30.1 micrograms/ml, yet almost 2-times over the reference value. These changes in CSF copper levels seem to be independent of the bacterial agent causing the meningitis. In conclusion, the CSF copper level can help to differential diagnosis of bacterial meningitis. Furthermore, the return of CSF copper to normal values occurs after 48 hours of antibiotic therapy, but is needed further investigations for elucidate the mechanisms involved in this process and the clinical implications for patients.

206B.—Copper Status Follow-on in Zinc-supplemented Infants with Fetal Malnutrition. Marcelo Maturana,* Guillermo Venegas,* Aldo Rodriguez[+] and Carlos Castillo-Duran. *Department of Pediatrics, Faculty of Medicine, University of Concepción, Chile, [+]Faculty of Pharmacolgy, University of Concepción, Chile, Food's Technology Institute, University of Chile, Santiago, Chile.

Zinc supplementation for Small for Age Infants improve the growth in terms of weight and height. However, the intestinal absorption of several trace elements could be competitive between them. With the aim of evaluate the copper status in Small for Gestational Age (SGA) infants supplemented with Zinc during first 6 month of life, we collect samples of plasma and hair of 25 SGA infants supplemented with oral Zinc Acetate (5 mg daily), and 27 SGA infants non-supplemented (placebo). Samples were collected at 30, 60, 90, 120 and 180 days of age, with informed consent of parents, and copper level was determinated with atomic absorption spectrophotometry. The general characteristics of both groups were similar in terms of Apgar scores, newborn weight (mean 2313 grs for Zinc-group and 2321 grs for placebo-group) and height (47.3 cms in Zinc group and 47.0 cms in placebo-group).

Results:

Hair copper (mcg/gr)	30 days	60 days	90 days	120 days	180 days
Zinc supplemented	25.4	30.5	26.3	25.7	27.5
Placebo supplemented	23.6	27.6	30.1	27.4	27.3
Plasma copper (mcg/ml)	**30 days**	**60 days**	**90 days**	**120 days**	**180 days**
Zinc supplemented	97.0	106.3	114.4	118.0	117.8
Placebo supplemented	92.1	104.8	115.4	120.1	127.5

Conclusions: Hair and plasma copper levels were normal in both groups, without stadistics differences between Zinc supplemented SGA infants and Placebo supplemented SGA infants. These data suggest that supplementation with Zinc in low dose

don't interfere with intestinal copper absorption and likely don't affect the nutritional status of copper.

207B.—Lipoprotein Metabolism and Putative Markers of Copper Status and Bone Health are Unaffected by Chronic Zinc Supplementation at the Upper Intake Level. Liadhan B. McAnena, Maxine P. Bonham, Jacqueline M. O'Connor, Paula Walsh, C. Stephen Downes, Bernadette M. Hannigan and J.J. Strain. Northern Ireland Centre for Diet and Health (NICHE), University of Ulster, Coleraine, Northern Ireland BT52 1SA.

High-dose zinc supplementation has repeatedly been associated with signs of copper deficiency. A developing culture of self-medication with mineral/vitamin supplements, along with increasing use of zinc supplements to alleviate symptoms of the common cold, has raised the issue of possible adverse effects of chronic zinc supplementation on copper status. Some known consequences of low copper status are bone abnormalities and decreased high-density-lipoprotein cholesterol (HDL). HDL has been decreased experimentally by low-copper diets and by zinc intakes of 50 mg/day (1). The US daily tolerable upper intake level of zinc intake (UL) is 40 mg zinc/day. The current double-blind intervention trial examined the interactions of zinc with copper status, bone health and lipoprotein metabolism. Healthy men ($n = 19$) took 30 mg zinc/day for 14 weeks followed by 3 mg copper/day for 8 weeks to counteract any potential harmful effects of zinc supplementation. Control subjects ($n = 19$) took placebo. Reported mean dietary zinc intake was 9.4 mg/day (SD $\pm$ 2.7). Blood sampling timepoints were weeks 0, 2, 14 (zinc supplementation endpoint), 16, 18 and 22 (copper supplementation endpoint). Copper status was assessed by serum copper, erythrocyte superoxide dismutase activity, and serum caeruloplasmin concentration and activity. Zinc status was assessed by the putative indices of serum zinc, alkaline phosphatase (ALP) activity and extracellular SOD concentration. Bone health was assessed by urinary catabolites of bone turnover (pyrimidine and deoxypyrimidine) and by serum osteocalcin. Serum HDL and total cholesterol and triglycerides were analyzed enzymatically and LDL-cholesterol was then calculated. A zinc intake totalling 40 mg/day was associated with a significant increase only in ALP activity ($P < 0.017$), after 14 weeks. Zinc supplementation at the UL, followed by copper supplementation, had no significant effect on any of the putative markers of copper status or bone turnover, nor on lipid profiles, over the three-month study period. These findings support recent guidelines that a UL of 40 mg zinc/day should pose no risk of adverse health effects for most individuals. This work was supported by funding from the Food Standards Agency (AN0553).

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209B.—Analyses of Menkes Disease Mutations in the MNK Copper ATPase Suggest its Copper-induced Trafficking is Associated with the Formation of a Phosphorylated Catalytic Intermediate. Michael J. Petris,* James Camakaris,[+] Ilia Voskoboinik,[+] Byung-Eun Kim,* Kathryn Smith* and Julian Mercer.[‡] *Nutritional Sciences, University of Missouri, Columbia, MO., +Department of Genetics, University of Melbourne, VIC, Australia, and [‡]Centre for Cellular and Molecular Biology School of Biological and Chemical Sciences, Deakin University, VIC, Australia.

The Menkes protein (MNK, ATP7A) is a copper transporting P-type ATPase that is defective in the X-linked recessive copper deficiency disorder, Menkes disease. The MNK protein is localized in the trans-Golgi network (TGN) of cultured cells where it transports copper to enzymes synthesized within secretory compartments. However, the protein also relocalizes to the plasma membrane and exports copper if cytoplasmic copper levels become elevated. This copper-regulated trafficking of MNK allows the protein to exist at appropriate levels at the TGN and plasma membrane, to fulfil its roles in the metallation of cuproenzymes and copper efflux. In this study, immunofluorescence microscopy was used to localize recombinant MNK protein expressed in transfected cultured human cells. Mutations preventing the formation of a phosphorylated catalytic intermediate were found to inhibit the copper-induced trafficking of the MNK protein from the TGN to the plasma membrane. However, mutations that promoted hyperphosphorylation of the enzyme resulted in a constitutive trafficking response and caused the accumulation of MNK at the plasma membrane in the absence of elevated copper. Our findings provide evidence for an association between the catalytic conformation of MNK and the sorting mechanisms controlling its trafficking. These studies reveal a novel mechanism for regulating the relative intracellular distributions of an ion transporter, which occurs as a function of its catalytic activity.

210B.—Prion Protein Expression Increases Cellular Copper Uptake. Walid Rachidi,* Pascal Guiraud,* Jacqueline Riondel,* Sylvain Lehmann,[+] Hubert Laude and Alain Favier.*** *LBSO, Faculté de pharmacie, Domaine de la Merci, 38706 la Tronche, France; [+]IGH, CNRS U.P.R 1142, 141 rue de la cardonille, 34396 Montpellier, France; **INRA, 78350 Jouy-En-josas, France.

It is believed that the agent responsible for Prion diseases or transmissible spongiform encephalopathies is a protein termed PrPSc. PrPSc is a conformational variant of the normal host protein, PrPC. Although PrPC function remains elusive. The amino terminal (N-terminus) of PrPC contains a series of octapeptide repeats possessing the following consensus sequence PHGGGWGQ. This region, amongst the most conserved regions of mammalian PrP, has been implicated in the binding of divalent metal ions, and in particular Cu. Due to the capacity of PrPC to bind copper, this protein has been implicated in Cu transport and metabolism and in the defense mechanism of the cell against oxidative insult, possibly through a regulation of the Cu/Zn superoxide dismutase activity (Cu/Zn SOD). However, a recent study may lead to a re-evaluation of these hypothesis since in transgenic models, link between PrP expression and Cu metabolism or SOD activities has not been confirmed. In this study, we report a link between PrPC expression in stable expressing tetracycline regulatable murine PrP gene in a rabbit epithelial cell line (RK13), copper uptake and resistance to oxidative stress. Radioactive copper (64Cu) was used at a physiological concentration to demonstrate that uptake

of copper in our cells is related to the level of PrPC expression. A pre-treatment of this cells over expressing PrP with phosphatidyl specific phospholipase C (PIPLC) significantly decreased the copper uptake. PrP cleavage after a 24 hours incubation with 64Cu led to the release of a PrP-64Cu complex in the medium. When increasing copper concentration in medium we observed a significant increase level of intracellular copper probably due to activation of endocytosis of copper via PrPC. Prion protein expression may be involved in both the transport of copper and resistance to oxidative stress.

211B.—Identification of New Copper-binding Proteins in Human Erythrocytes: Possible Implications in Risk Assessment. Hernán Speisky,*+ Paola Navarro and Inés Jiménez.*+ *Nutritional Toxicology Unit, INTA, +Faculty Chemical & Pharmaceutical Sciences, University of Chile, Santiago, Chile (hspeisky@uec.inta,uchile.cl).

The identification and characterization of new copper-binding proteins (CuBP) represents a valuable approach in the search for putative biomarkers of over-exposure to copper. CuBP play a fundamental role in Cu homeostasis by preventing the redox-activity and electrophilic character of free Cu ions, and by securing an adequate transport, delivery, storage and/or excretion of the metal. Since portal plasma Cu is partly taken up by circulating cells, we have focused our interest in assessing the capacity of proteins present in human erythrocytes (HE) to sequester copper. The incubation of such cells in the presence of (1–50 M) of 64Cu (as CuSO4-Histidine) is followed by a time- and concentration-dependent uptake of the isotope. Experiments using [3H]-His indicate that the entry of Cu and His into the HE is independent. Under near-maximal 64Cu incorporation conditions (20 min incubation), about two-thirds of the cpm added to the suspension were recovered in 20,000xg supernatants (S-20). 64CuBP in S-20 were subjected to separation by successive size exclusion and metal-affinity chromatography. Most (90%) of the radioactivity loaded into a Sephadex G-75 column was recovered in association with molecules of MMr greater than 60 KDa (largely hemoglobin). Less than 1% was associated with metallothionein or superoxide dismutase. Further purification of the high-MMr 64Cu-binding fractions was attained in a Sephadex G-200 column whose elution profile revealed two major peaks. The cpm/g protein ratio of the first peak (higher MMr) was proportional to the concentration of Cu presented to the HE. The second peak corresponded to Hb. Proteins from the first peak were concentrated in a Hi-Trap mini-column (suited to trap high-affinity CuBP), eluted with imidazole, and applied into a Sephacryl S-300 column. A hemoglobin-free peak (MMr $>$ 200 KDa) was obtained. PAGE analysis (5–10%) of such peak, conducted under native or SDS conditions, revealed the existence of two low MMr bands (aprox. 30 and 40 KDa). Incubation of the S-300 peak in the presence of 64Cu revealed a saturable, concentration-dependent, and EDTA-removable binding of copper. Based on the estimated affinity (KD = 20 M), capacity (2 g-at./mol), and ability of this HE-occurring protein to bind a broad range of copper concentrations, its study as a putative biomarker of copper over-exposure appears warranted. Supported by Fondecyt # 1010705.

212B.—In Vitro Formation of a Copper-homocysteine Complex: Potential Redox Implications. Hernán Speisky,*+ Paola Navarro, Claudio Olea-Azar+ and Inés Jiménez.*+ *Nutritional Toxicology Unit, INTA, +Faculty Chemical & Pharmaceutical Sciences, University of Chile, Santiago, Chile (hspeisky@uec.inta,uchile.cl).

Homocysteine (HC) may be implicated in the progression of various oxidative stress-related vascular and neurodegenerative diseases. Due to the presence of an −SH group on its structure, HC and other thiols such as cysteine (C) may exert a double-edge action on the cell's redox status. Depending on whether Cu ions occur concomitantly or not, these thiols could either promote or prevent free radical-generation and its consequences. We have addressed in vitro the interaction between HC and Cu, in terms of the consequences that such interaction may have on the free radical-scavenging properties of HC and on the redox activity of the metal. The bleaching of ABTS·+, a color-stable free radical, was employed to assess the free radical-scavenging capacity of HC. While HC concentration-dependently (0–10 μM) bleached ABTS·+, its pre-incubation with Cu2+ ions led to a metal concentration-dependent (0.1–2.5 μM) decrease in the bleaching capacity (BC). At a Cu:HC ratio of 1:3, the BC of a pre-formed mixture was lowered but only to one third of that of HC alone. However, further additions of Cu resulted in no greater decreases of BC. Under similar conditions, the BC of C was, in turn, completely lost. Mixtures of Cu plus HC exhibited BC that were proportional to their thio-reactivity. Increasing concentrations of Cu plus HC (at a fixed 1:1 ratio) led to linear and concentration-dependent increases in BC. Addition of TRIEN, EDTA or histidine to a pre-incubated Cu plus HC mixture failed to affect its BC. Yet, the incubation of Cu with TRIEN or EDTA (but not histidine) prior to HC addition, totally prevented the loss of BC induced by Cu. EPR, bathocuprein (which binds Cu+) and ascorbate-oxidation studies indicate that HC promotes the reduction of Cu2+ ions, bringing the redox stabilization of copper under a Cu(I)-HC complex form. Thus, in the presence of oxygen, HC reduced Cu2+ ions without promoting the superoxide-dependent cytochrome c reduction. Data support the contention that: i) at concentrations found in plasma, Cu and HC readily interact forming a Cu-HC complex; ii) such interaction implies lowering but only to one-third the original free radical scavenging capacity of HC; iii) the thio-reactivity of the complex is proportional to its BC; iv) the complex formed be stable to the presence of some copper-chelators. It is suggested that, if occurring in vivo, an interaction between Cu and HC may lead to stabilizing Cu ions under a redox-inactive form capable of partially retaining the free radical scavenging properties of homocysteine. Supported by Fondecyt # 1010705; DID I008-99/2.

213B.—Six Month Follow-up of Copper Status in Small for Date Newborns. Venegas, G., Maturana, M., Rodríguez, A. and Castillo, C. Pediatrics Dep. and Hospital University of Concepción- Chile.

Back Ground – Aims: Over the past ten years several studies have measured copper (Cu) levels in newborn and infants until a sensitive method for the assessment of Cu status is available in clinical we must use the plasma and hair levels as a reference Cu status in small for date newborns (S.F.D.) has received scant

attention. In South American SFD babies are highly prevalent and associated with morbid-mortality and long term repercussions. The aim of this study was to follow-up the Cu status in SFD newborns in the first six months of life. Methods: In 63 SFD newborns aged 12–24 hours a venous blood was withdrawn and a hair sample was taken. Babies with congenital malformations, asphyxia or perinatal infections were excluded as well as pre-eclamptic mothers or intra hepatic cholestasis. Same samples were collected at 30, 60, 90 and 180 days of age. Cu determination was made by means of Atomic Absorption Spectrophotometry. Results: SFD average weight was 2302 g (1400–2610) and height was 47 cm (41–51 cm). Cu hair and plasma levels at birth and 30, 60, 90, 180 days, was as follows: 18,57 ug/g and 57,8 ug/dl; 24,4 ug/dl and 94,4 ug/dl; 28,9 ug/g and 105,5 ug/dl; 28,4 ug/g and 114,9 ug/dl; 27,4 ug/g and 122,0 ug/dl respectively. Discussion: Serum and hair Cu status are within normal range in these SFD babies in relationships our previous reports in healthy infants and newborns. Follow-up 30, 60, 90, 180 days bloods levels increase steadly whereas Cu hair rise slightly up to 60 days plateaning thereafter.

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2. Maturana M., Venegas G., Castillo C. (1997) "Hair and plasma Copper in fetal malnutrition newborns and his mothers". Pediatric, vol. 18 No 3, pag. 20–24.

214B.—Molecular Definitions of Yeast Physiological and Toxic Copper Levels. Yongxing Wang, Jose Heredia and Zhiwu Zhu. Department of Environmental Toxicology, University of California, Santa Cruz, CA 95064.

The intracellular copper concentration needs to be balanced at its physiological level to simultaneously avoid both deficiency and excess, because copper in living organisms is essential and toxic depending on its concentration. We are interested in not only determining the exact concentrations of physiological and toxic copper levels, but also how these two copper levels are molecularly defined. We have discovered that the yeast copper-sensing transcription factor Mac1p, which regulates the genes encoding high affinity copper ion transporters CTR1 and CTR3, is able to sense two different copper levels. The Mac1p senses the physiological copper level through a conserved 'Cu-fist' in the N-terminal DNA-binding domain and the toxic copper level through a unique REP-I in the C-terminal activation domain. We have obtained evidence indicating that the physiological and toxic copper levels are defined molecularly by the copper ion-binding affinities of the two motifs, respectively. We have also discovered that yeast copper uptake is copper-dependent. This dependence likely prevents intracellular copper ions from falling below the physiological level. The role of Mac1p in yeast copper ion homeostasis by controlling copper uptake will be discussed.

215B.—The Effect of Molybdenum or Iron on Copper Status of Growing Lambs. Claire L. Williams, Robert G. Wilkinson and Alexander M. Mackenzie. ASRC, School of Agriculture, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK.

Copper (Cu) deficiency in ruminant animals is known to be induced by the dietary antagonists molybdenum (Mo), Iron (Fe) and sulfur (S). However, accurate assessments of copper status of ruminants has been problematic. The objective of this study was to assess the effect of molybdenum or iron in the presence of sulfur on the copper status of intensively reared lambs. Forty-eight individually penned Charolais cross female lambs were randomly allocated to one of four dietary treatment groups and fed an ad libitum complete basal diet containing 5.47 mg/kg DM Cu and 1.29 mg/kg DM Mo. Group one received no additional mineral supplementation (control), group two received an additional 500 mg/kg Fe and 2 g/kg S (Fe), group three received an additional 5 mg/kg Mo and 2 g/kg S (5 Mo) and group four received 10 mg/kg Mo and 2g/kg S (10 Mo). Blood samples were taken once weekly by jugular venepuncture for subsequent analysis of plasma copper (Pl-Cu), haemoglobin (Hb), haematocrit (Hc), ceruloplasmin (CP) and superoxide dismutase (SOD). The ceruloplasmin to plasma copper ratio (CP:Pl-Cu) was determined to assess copper status. All lambs were slaughtered after ten weeks and liver copper concentrations were analyzed. The CP:Pl-Cu ratio indicated a significant difference between treatments ($p < 0.001$) from week 1 onwards, with the 10 Mo treatment group having a lower ratio compared to the 5Mo, Fe and control groups respectively. The 10 Mo group had the lowest mean liver copper concentrations at slaughter (58.8μg/g DM) compared to the 5 Mo (76.2 μg/g DM), Fe (93.2 μg/g DM) and control (278.1 μg/g DM) groups. There was no significant difference between treatments for Hc, Hb or SOD. The CP:Pl-Cu ratio decrease in both the Fe and Mo supplemented groups indicates that the ratio is sensitive to dietary copper antagonists. High dietary molybdenum may enhance thiomolybdate (TM) production which binds copper within the rumen, but once absorbed into the blood or tissues, may induce systemic effects and inhibit copper enzyme activity. This may explain the reduced CP activity in the Mo treatment groups compared with the Fe or control. Therefore, the CP:Pl-Cu ratio is sensitive to the antagonistic effects of Mo in the diet of ruminants and may provide a method to assess copper status.

216B.—Effect of Three Different Pork Meat Protein Fractions on Nonheme Iron Absorption in Humans. Sussi B. Bæch,* Marianne Hansen,* Klaus Bukhave, Tine Eriksen,$^{+}$ Peter P. Purslow,$^{++}$ Leif H. Skibsted^{+} and Brittmarie Sandström.*** LMC Center f. adv. Food Studies/ *Res. Dept. Human Nutr., $^{+}$Dept. Diary and Food Sci., Royal Vet. Agric. Univ., Frederiksberg, Denmark **Section of Biochem. Nutr., Biocentrum-Techn. Univ. Denmark, Lyngby, Denmark and $^{++}$Dept of Biol. Sci., Univ. Stirling, UK.

Introduction: Meat tissue is known to enhance nonheme iron absorption in humans, 1,2 however, knowledge about the nature of the promoting substances of meat is lacking. Objective: To investigate the effect of water- (ws) salt- (ss) and insoluble (is) pork meat proteins on nonheme iron absorption. Method: Seventeen healthy women (23 ± 3 y) were served a basic meal (rice and vegetables) (A) and three different meat protein meals (basic meal + 12 g of ws-, ss- or is meat proteins) (B, C and D). The basic meal contained 2.3 mg nonheme iron and 800 mg (1.3 mmol) phytate. The meals were served twice in random order on four consecutive days in two different periods, e.g.: ABBA (period 1) and CDDC (period 2). The meals were extrinsically

labeled with 55Fe or 59Fe and nonheme iron absorption was determined from the activity of 55Fe and 59Fe in blood samples 18 days after the last meal. The protein fractions were purified by a): water extraction and filtration (ws proteins) b): a + extraction in Phosphate buffered saline (PBS) (0.3 M NaCl, 0.05 M Na2HPO4 and 0.1 M NaH2PO4), filtration and precipitation (ss proteins) and c): b + extraction in PBS, filtration, extraction in water and filtration (proteins). Results: 12 g of proteins enhanced the nonheme iron absorption by 63% (ws proteins) ($p < 0.001$), 110% (ss proteins) ($p < 0.001$) and 41% (is proteins) ($p < 0.1$) compared to the basic meal. Discussion: The ws- and ss protein fractions seem to be nonheme iron absorption promoting. The lack of effect with the same amount of proteins in the insoluble protein fraction suggests that protein content per se is not nonheme iron absorption promoting.

1. Martinez-Torres C, Layrisse M. (1971) Iron absorption from veal muscle. Am J Clin Nutr 24:531–40.
2. Cook JD, Monsen ER. (1976) Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 29:859–67.

217B.—Day-to-day Variation in Iron Status Parameters in Young Iron Deplete Women. Anita Belza,* Annette K. Ersbøll,+ Shakuntala H. Thilsted S* and Marianne Hansen.* *Research Department of Human Nutrition/LMC Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, +Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Denmark.

Background. Assessment of iron status in intervention studies is often based on a single blood sample. The reliability of results is influenced by biological and analytical variation. If the day-to-day intra-individual variation of a biochemical measure is large, the assessment of iron status of an individual can be misinterpreted if only one blood sample is taken. On the other hand, it is important to limit the number of blood samples to the minimum required in order to reduce iron losses, the cost of analyses, the time consumption and subject discomfort. Previous methodological studies have suggested that 1–3 blood sampling days may be required for estimating serum ferritin, a frequently used iron status parameter. Unfortunately, the above studies were insufficiently designed regarding the standardisation of medication, smoking and consumption of food and drink (including alcohol) in the subjects. Aim. The objective of the present study was to optimise the methodology for estimation of iron status by determining the day-to-day variation of five iron status parameters in a more controlled study design. We aim to assess the required amount of blood sample days needed in order to determine the "true value" of each blood parameter. Subjects and methods. Venous blood samples were collected from 12 women (23–30 years) with low iron stores (12–30 μg serum ferritin/l). Blood samples were collected under standardised conditions on 15 non-consecutive days during a 5-weeks' period. All blood samples were analyzed for haemoglobin (Hb), serum ferritin (SF), reticulocytes (RET), red blood cell volume distribution width (RDW), and serum transferrin receptors (TfR) by enzyme immunoassay (EIA) and immunoturbidimetric assay (IDeA). Results. When analytical variations had been accounted for, the day-to-day variation (CV%) of each blood parameter was estimated to: Hb (3.0%), SF (14.8%), RET (28.2%), RDW (2.4%), TfREIA (9.9%) and TfRIDeA (8.1%). The required number of blood sample days needed to determine the "true value" of each parameter (within 20% accuracy with 95% confidence) was 1 day for Hb, RDW, TfREIA and TfRIdeA. For SF and RET, 2 and 8 days, respectively, were required. Conclusion. As serum ferritin is a main parameter for estimation of iron status, the present data suggested that two blood sampling days are required in order to determine the iron status in intervention studies. At present, the use of reticulocytes seem less useful as an iron status parameter due to the very large day-to-day variation.

218B.—Daily Dietary Fe, Zn, and Cu Intake of Toddlers, 2 to 3 Years of Age, Living in Belgium (Western Europe). Douwina Bosscher, Harry Robberecht, Micheline Van Caillie Bertrand and Hendrik Deelstra. Dept. Pharmaceutical Sciences, Lab. Food Sciences, University of Antwerp (UIA), Antwerp, Belgium.

Preschool children are one of the most-affected groups for iron (Fe)- and zinc (Zn)-deficiencies. Estimates claim that about 17% of children in industrialized countries are victims of Fe-shortage. Based on data from the National Health and Nutrition Examination Survey III, only 19% of children between 1 and 3 years of age revealed adequate Zn intakes. Less is known about copper (Cu)-deficiency, especially in children, however earlier dietary Cu-levels have been found to be below the required Cu daily intake values. In this study daily dietary Fe, Zn, and Cu-intake in healthy toddlers in Belgium, aged 2 to 3 years, have been evaluated by duplicate portion sampling ($n = 115$). The elemental content was analyzed by atomic absorption spectrometry after acid destruction and heating in a microwave oven. Mean iron intake (4.8 ± 0.2 mg/day) was comparable with values found in other European countries (e.g. The Netherlands, U.K. and Northern Greece) but lower than values found in Boston (U.S.A.) and, far below the population reference intake (PRI) for Belgium (10 mg/day) and the RDA-value (7 mg/day). For Zn, mean intake (7.5 ± 2.6 mg/day) was higher than in other European countries, e.g. The Netherlands, U.K., Sweden (Helsingborg), and U.S.A. and, above the recently revised PRI for Belgium (4 mg/day) and the RDA (3 mg/day). The mean intake of Cu (0.7 ± 0.2 mg/day) was found to be similar to those found for most other countries, e.g. Australia (Brisbane), Finland (Helsinki), Germany (Duisburg) and The Netherlands, and was far above the PRI (0.4 mg/day) and the RDA (0.3 mg/day) for this element. The intake of Cu and Zn by the healthy toddler population in Belgium (Antwerp) seems to be adequate, while intake of Fe is critically low.

219B.—Influence of Wheat Bran, Cellulose and Phytic Acid on Fe- and Zn-availability from Weaning Foods (In Vitro). Douwina Bosscher, Rudy Van Cauwenbergh, Micheline Van Caillie Bertrand and Hendrik Deelstra. Dept. Pharmaceutical. Sciences, Lab. Food Sciences, University of Antwerp (UIA), Antwerp, Belgium.

Some sources of dietary fiber, especially those containing phytic acid (PA) (e.g. wheat bran), decrease trace element absorption but the relative roles of isolated dietary fibers versus PA remain

unresolved. In healthy infants, bioavailability of trace elements is difficult to study because of both methodological problems and ethical constraints. Therefore, we optimized and validated[1] an in vitro method to determine trace element bioavailability (availability). We used this model to study the effects of wheat bran (whole food), a-cellulose (isolated dietary fiber-fraction) and PA (isolated fraction)[2] on the availability of Fe and Zn in green beans. Green beans were lyophilized and mixed with 25 g wheat bran/100 g, 0.47 g cellulose/100 g, and 0.47 g PA/100 g. Element availability was measured by a continuous-flow-dialysis technique and was used as an index of bioavailability. Elemental content was quantified by atomic absorption spectrometry. PA contents were analyzed by HPLC. Incorporation of wheat bran into green beans lowered availability of Fe from 12.2 ± 0.3% to 1.2 ± 0.2% and of Zn from 23.8 ± 2.2% to 9.6 ± 1.0% ($P < 0.05$). Addition of cellulose reduced Fe- (3.78 ± 0.5%, $P < 0.05$) but not Zn-availability (28.0 ± 2.4%, $P > 0.05$). PA decreased Fe-availability to 2.6 ± 0.4% and Zn-availability to 12.3 ± 0.2% ($P < 0.05$). The reduction of Fe- and Zn-availability in green beans is smaller after addition of PA than after addition of wheat bran, despite the lower PA content of the wheat bran preparation. Compared to the cellulose-fraction, the PA content of wheat bran has a major inhibiting effect on Fe- and Zn-availability, however; also other substances present in bran contribute to this effect.

1. Bosscher et al. (2000) J. Ped. Gastroenterol. Nutr. 30:373–8.
2. Bosscher et al. (2000) Brit. J. Nutr. 86:241–7.

222B.—StableCalc. Software for Use in Inorganic Stable Isotope Studies. Jack R. Dainty. Institute of Food Research, Norwich, UK.

There are an increasing number of inorganic stable isotope experiments being undertaken and correct study design and interpretation of mass spectrometry data are vital if the results are to be useful for the wider nutrition community. The software tool described here has been developed to assist these tasks in a user-friendly environment. Studies involving labeled copper, zinc, calcium, iron, selenium and magnesium can be analyzed using the software and all the major experimental techniques are included as options. There are three calculation choices: 1) Quantity of dose required for a given experiment. This will depend on factors such as which isotope in the dose is enriched and by how much, which biological samples are being collected, the length of time sampling will continue and the precision with which the mass spectrometer can measure certain isotope ratios. 2) Quantity of each mineral source in a biological sample. Isotope ratios can be entered into StableCalc and an estimate will be made of the fraction of each mineral source present in the sample along with the uncertainty of each estimate. Knowledge of the total mineral content will then allow full quantification of all the sources present. 3) Quantity of dose that has been absorbed. If measurement uncertainties are known, a full error propagation option is available which can inform the researcher about the precision of their experiment.

224B.—Genetics of Iron Deficiency in Arabidopsis thaliana. Laura S. Green and Elizabeth E. Rogers. Department of Nutrtional Sciences, University of Missouri, Columbia, MO, United States.

In response to iron deficiency, all plants except the grasses induce Fe(III) chelate reductase activity, Fe(II) transport activity and proton release into the rhizosphere. Previously, we identified an Arabidopsis mutant, frd3, that constitutively expresses all three of these iron deficiency responses and overaccumulates iron, manganese, and zinc. The FRD3 gene is expressed in roots and is predicted to encode a membrane protein belonging to the multidrug and toxin efflux (MATE) family. It is tempting to speculate that FRD3 encodes a regulatory factor involved in sensing and/or responding to iron levels in Arabidopsis. A number of experiments are underway to characterize this novel protein and identify its role in iron nutrition. In order to identify other genes that act with FRD3 to regulate iron deficiency responses in Arabidopsis, we are currently screening for extragenic suppressors of the frd3 mutant phenotype. The excess iron and other metals in frd3 mutants give the plants a chlorotic appearance. A well-backcrossed line of frd3–3 has been mutated with EMS. M2 seeds are being screened for individuals who have lost the chlorotic phenotype of the frd3–3 parent. To date, a number of putative mutants have been identified. They are being rescreened for chlorosis, inducible iron deficiency responses and iron content.

225B.—The Effect of Iron Fortified Rye Bread Produced with Phytase on the Iron Status of Young Women. Hansen M.,* Belza, A.,* Thomsen, A.D.,+ Lindeløv, T.+ and Sandström, B.+ *Research Department of Human Nutrition/LMC Centre for Advanced Food Studies, The Royal Veterinary and Agricultural University, Denmark, +The Research and Development Department, Schulstad Bread A/S, DK-2650 Hvidovre, Denmark.

Background. Iron deficiency is a very common problem among young women and children. For example [a]40% of young Danish women have low or depleted iron stores and up to 8% of the younger women are anaemic. This is mainly ascribed to a low iron intake and a low bioavailability of dietary iron. Iron fortification of flour and bread is common in several countries but its efficacy has been questioned due to a low bioavailability of the fortification iron used and/or a high phytate content of the diet. The phytate content of bread may be reduced by addition of phytase during the bread preparation. A previous intervention study with young women has shown that daily intake of iron fortified rye bread for 5 months did not improve iron status but prevented a decrease in iron stores as observed in the control group given unfortified rye bread. The fact that no actual increase in iron stores was observed may have been due to the phytate content of the bread. Objectives: To study the effect of using phytase in the production of iron-fortified rye bread on iron status of iron deficient women. Subjects and methods: In a randomized single blinded parallel intervention study, two groups of iron deficient non-anaemic women (20–38 years) were given 142 g rye bread fortified with 7.7 mg iron as ferrofumerate/100 g (total iron 9.4 mg/100 g) daily for 5 months. The women were randomized to receive bread produced with ($n = 23$) or without ($n = 22$) phytase with their habitual diet. Iron status was followed in blood samples collected at 0, 2.5 and 5 months. Dietary records were made before and during the intervention.

Results: The total phytate content (SIP3–6) was 23 μmol/100 g in the phytase-produced bread and 130 μmol/100 g in the control bread. The phytase seemed to selectively cleave the IP3 (99% reduction) and IP4 (93%) whereas IP5 (40%) and IP6 (33%) was not as efficiently reduced. Compliance was approximately 97% in each group. Iron intake increased from 11 mg/d before the intervention to 27 mg/day during the intervention period. Intake of dietary fiber also increased from 23 to 30 g/day. The concentration of serum ferritin at baseline and after 5 months intervention was 17.8 [15.2;20.8] μg/l (geometric mean, 95% CI) and 18.2 [15.2;21.6] μg/l in the intervention group ($p = 1.0$), and 14.9 [12.4;17.9] and 17.2 [13.7;21.4] μg/l in the control group, ($p = 0.1$). The concentration of haemoglobin and reticulocytes did not change in any of the groups either. Conclusions: In conclusion, there was no significant change in iron status the two study groups, indicating that there was no special benefit of the use of phytase. Iron status was, however, maintained in both groups as opposed to the decrease observed in subjects intervened with unfortified bread in a previous study. As the IP5–6 content of the breads was rather low, the composition of the habitual diet of the subjects may be responsible for a low utilization of the fortification iron. The data suggest that there is a need for testing iron compounds protected from phytate and other inhibitors of iron absorption.

226B.—The Beneficial Effect of Combined Iron and Zinc Supplementation on Anemia in Indonesian Infants 4 to 7 Months of Age. Adi Hidayat,* Budi Utomo,[+] Sunawang and Michael J. Dibley.[++]** *Medical Faculty, University of Trisakti, Jakarta, Indonesia, [+]Center for Health Research, University of Indonesia, Jakarta, Indonesia, **UNICEF, Jakarta, Indonesia, [++]School of Population Health, Faculty of Medicine and Health Sciences, University of Newcastle, Newcastle, Australia.

Anemia resulting from insufficient iron for synthesis of hemoglobin is the most common nutritional disorder among children in the developing world. Furthermore, adequate zinc nutrition is difficult to attain during infancy in many developing countries. A community-based, double-blind, controlled community trial was conducted to determine the beneficial effect of combined iron and zinc supplementation on anemia during infancy. Infants aged 4 to 7 months of age were assigned randomly to receive one of four types of supplements: 10 mg of zinc as zinc sulfate ($n = 200$), 10 mg iron as iron sulfate ($n = 200$), 10 mg zinc combined with 10 mg iron ($n = 200$) or placebo ($n = 200$) five times a week for 6 months. Prior to supplementation, all infants were given 100,000 IU of vitamin A. Infants predominantly breastfed and free from apparent congenital anomalities were included in the trial. Hemoglobin was measured from a capillary heelprick by the Hemocue (Hemocue AB, Angelholm, Sweden) at baseline and at the end of supplementation. Family socioeconomic and demographic characteristics were also obtained. There were no differences in baseline charactersitics between the four treatment groups. After 6 months of supplementation the mean hemogobin of infants in the iron alone (Hb = 10.9 gm/dL), and combined iron and zinc group (Hb = 10.8 gm/dL) was significantly higher ($p < 0.01$) than the hemoglobin of the infants in the zinc alone (Hb = 10.0 gm/dL), and placebo (Hb = 10.2 gm/dL) groups. The iron alone, and combined iron and zinc supplementation reduced the prevalence of anemia by 29.7% and 21.4% respectively. Supplementation with zinc alone increased the prevalence of anemia by 15.3%. This is particularly important because iron and zinc supplementation is given to infants at the earliest age to prevent systemic abnormalities caused by deficiency of these micronutrients. A study in Mexico showed that in preschool children after 12 months of supplementation with 20 mg iron alone, or 20 mg iron and 20 mg zinc, or 20 mg zinc alone, or placebo, the hemoglobin concentration increased in all groups. In this study, the hemoglobin concentration decreased in the groups with non-iron (zinc and placebo) supplementation. In areas where iron deficiency is a common problem, zinc supplements alone for six months in infants may have a negative impact on hemoglobin levels and highlights the need for caution with single nutrient supplements in infants.

228B.—Two Forms of Ferritin in Serum and Secreted by Hepatoma Cells are Differentially Regulated by Iron and Inflammatory Hormones. Christine L. Juska, Phillip P.V. Nguyen, Gloria Kwan, Rashmi Malpe, Julia Truty and Maria C. Linder. California Sate University, Fullerton, CA, USA.

Serum ferritin is defined as plasma protein that reacts with specific antibody against intracellular liver and spleen ferritins, but has not been well characterized. Assays of serum ferritin are used to assess body iron stores. However, other physiological conditions, notably inflammation, increase serum ferritin levels without altering body iron. Previously, we reported that a rat hepatoma cell line (H4-II-E-C3) secreted ferritin into the medium in response to iron and inflammatory cytokines. Using this cell line, we isolated and characterized the ferritin, comparing it to ferritin isolated from human and horse serum as well as intracellular hepatocyte ferritin. We also studied its regulation by iron and cytokines. Two forms of secreted ferritin were isolated from the conditioned medium and also detected in serum. One (A) was indistinguishable from intracellular ferritin [with H and L subunits (and amino acid sequence), Mr of 490 k] except that it contained much less iron. The other form (B) was 140 kDa, had subunits of 48–52 kDa, little or no iron, was glycosylated, and bound to protein G but not protein A. Iron enhanced the rate of synthesis of both forms of secreted ferritin, but for B, regulation was transcriptional, while for A it was translational. Cytokines only enhanced synthesis of B. Thus, serum ferritin is a complex mixture of secreted proteins encoded by several genes under differential regulation.

229B.—Evaluation of Cool Plasma ICP-MS for Fe Isotope Ratio Measurements for Fe Tracer Studies. Peter Kastenmayer and Jennifer Clough. Nestlé Research Center, Lausanne, Switzerland.

Stable isotope tracer techniques are useful tools for studying Fe metabolism in humans. Depending on the study design, several tracer isotopes (54Fe, 57Fe, 58Fe) have to be accurately quantified in metabolic samples. Purpose of this work was to evaluate the use of quadrupole ICP-MS (Q-ICP-MS) in the cool plasma mode for measuring the isotopic ratios 54Fe/56Fe, 57Fe/56Fe and 58Fe/56Fe. Isobaric overlap (e.g. 58Ni on 58Fe) and interferences of polyatomic ions (e.g. 40Ar16O+,

40Ar16OH+) on 56Fe and 57Fe are well known to cause problems to accurate isotope ratio measurements in Q-ICP-MS. Under cool plasma conditions, interferences caused by Ar can be effectively reduced. Measurements on samples containing high amounts of Ca (e.g. feces, blood) showed however that, in order to control interferences by 40Ca16O+ and 40Ca16OH+, a pre-separation step from matrix elements using anion exchange chromatography was required. Therefore, it was decided to restrict isotope ratio measurements to pure Fe solutions. The instrument used was a Perkin-Elmer ELAN 6000 with a cross flow nebuliser and a Scott-type double pass spray chamber. RF power was reduced to 500 W, which decreased the 40Ar16O+ signal to <100 cps. Samples were diluted to a concentration of 500 ppb Fe in 0.1 M HNO3. Instrumental mass bias was corrected for by measuring the isotope ratios of a Fe standard solution (Merck) with natural isotopic composition recalibrating every 10 samples. Mean within run precision (5 replicates) was 1% for 54Fe/56Fe and 57Fe/56Fe and 1.2% for 58Fe/56Fe. Repeatability determined by measuring 500 ppb Fe standard solutions over 3 weeks was 0.5% for 54Fe/56Fe and 57Fe/56Fe and 1.0% for 58Fe/56Fe (n = 32). After correction for mass bias all isotope ratios were within 0.6% of the accepted IUPAC values. Accuracy was also verified by spiking standard solutions with known amounts of enriched 54Fe, 57Fe or 58Fe. Good agreement was found between calculated and measured enrichments. Limits of detection for enrichments of 54Fe, 57Fe or 58Fe in samples calculated based on the average with-in run RSD were 1.3% for 54Fe and 57Fe and 1.6% for 58Fe. Fe isotope ratios of fecal samples from a metabolic study were measured and results were compared to measurements by quadrupole thermal ionization mass spectrometry (Q-TIMS). Agreement between the two methods was excellent. Mean difference of measured isotope ratios was <1.4% (n = 20) for all ratios. In conclusion, Q-ICP-MS operated in the cool plasma mode can be used to analyze for stable isotope of Fe.

231B.—Intracellular Iron Content is Increased during Cellular Aging and Reversed by N-t-butyl Hydroxylamine Treatment in Human Fibroblasts. David W. Killilea, Hani Atamna and Bruce N. Ames. Department of Molecular and Cellular Biology, University of California, Berkeley CA and Children's Hospital Oakland Research Institute, Oakland, CA.

Normal fibroblasts serve as a model of in vitro senescence; with aging, doubling time, cellular size, and sensitivity to oxidative stress is increased while mitochondrial and lysosomal function is decreased. N-t-butyl hydroxyl mine (N-tBHA), the hydrolysis product of the spin-trap -phenyl-N-t-butyl nitrone (PBN), delays senescence, reverses mitochondrial decay, and lowers oxidative stress [Atamna et al, J. Biol. Chem. 275:6741, 2000 and Atamna et al, FASEB J 15:2196, 2001]. Loss of iron homeostasis occurs in aging and age-related diseases; thus we hypothesized that the senescence-delaying activity of N-tBHA would also restore iron homeostasis. To test this hypothesis, we maintained human lung fibroblasts (IMR-90) until senescence with and without N-tBHA or O-t-butyl hydroxylamine (O-tBHA, inactive isoform of N-tBHA). The cells were analyzed at different intervals for total iron and other physiologically relevant metals using inductively-coupled plasma spectrometry, and for senescence-related markers, such as cellular volume and beta-galactosidase activity. We found a 10–20 fold senescence-dependent increase in cellular iron in control (non-treated) cells. This finding is consistent with reports of an age-dependent increase of tissue iron content in vivo. Additionally, we see a smaller 2–4 fold senescence-dependent increase in intracellular calcium, magnesium, and zinc. N-tBHA dose-dependently attenuated the senescence-dependent increase of iron as well as the other metals, while O-tBHA had no effect. Interestingly, a senescence-associated increase in cellular volume and increase in senescence-associated beta-galactosidase activity were also dose-dependently delayed by only N-tBHA. These data support IMR-90 fibroblasts as a relevant model to study cellular aging and senescence, as they undergo similar age-related changes in iron homeostasis as reported in vivo. These data also indicate that altered metal homeostasis—especially iron—is a component of the cellular senescence paradigm in cultured fibroblasts. Additionally, N-tBHA may exert its senescence-delaying effects in fibroblasts in part by restoring iron homeostasis and thus reversing major senescence-associated changes in cellular physiology and oxidative stress levels.

232B.—Efficiency of Iron Supplementation as Affected by Cadmium in Anaemic Rats. Krejpcio, Wójciak and Olejnik. Department of Human Nutrition and Hygiene, August Cieszkowski. Agricultural University, Poznan, Poland.

Toxicity of Cd depends on various factors, including its interactions with some nutrients, especially Fe and its nutritional status. Since Fe deficiency is fairly common in adult women we investigated the effect of oral Cd administration on hematologic parameters and the iron body status of female anaemic rats. Materials and Methods:Anaemic and Fe-sufficient female Wistar rats were fed diets supplemented with and without Fe salts (ferrous lactate and gluconate: 10 mg Fe/kg b.w./day) with and/or without concomitant CdCl2 (2 mgCd/kg b.w./day) for 10 consecutive days. At the end of experiment rats were sacrificed to collect blood (by heart puncture) and harvest liver and kidneys for biochemical analyses. Biochemical tests in blood included: Hb, Hct, RBC, serum Fe (Fe-S) and TIBC that were determined with the use of the Pointe Scientific kit reagents. The content of Fe in the liver and kidneys (Fe-L, Fe-K), after wet digestion of samples, was analyzed by flame AAS method. The accuracy and precision for Fe determinations were assessed by multiple analysis of the reference material (Bovine liver, CRM 185, Brussels). For statistical evaluation of the results, ANOVA and Tukey tests and linear regression analysis were applied at $P < 0.05$. It was found that hematologic parameters (Hb, Hct, RBC) and Fe-status parameters (Fe-S, TIBC, Fe-S, Fe-L and Fe-K) depended on dietary Fe. Supplementation of diets with ferrous lactate and ferrous gluconate significantly increased Fe body stores in the anaemic rats. Oral Cd administration affected the Fe status in the Fe-deficient rats leading to a decrease of Fe absorption and metabolic utilization of this element. The hemoglobin regeneration efficiency was significantly decreased by Cd in the anaemic rats supplemented with both Fe salts in comparison with the respective groups without Cd. The bioavailability of Fe from lactate and gluconate was equally decreased by small doses of oral Cd. We conclude that small doses of oral Cd disturb Fe absorption in the rats and the Fe-Cd interaction that takes place in the gastrointestinal tract is decisive for Fe metabolic utilization in animals. It is suggested that chronic oral cadmium

intoxication diminishes beneficial effects of iron supplementation in anaemic animals and most probably in humans.

233B.—Effectiveness of Phytase as a Novel Tool in Improving Dietary Iron Bioavailability. Xin Gen Lei, Chad H. Stahl and Jesus M. Porres. Department of Animal Science, Cornell University, Ithaca, NY.

Bioavailability of dietary iron to simple-stomached animals and humans is severely affected by phytate. Although supplemental phytase has been effectively used in diets for food animals to improve utilization of phytate-bound phosphorus and other minerals, the potential value of phytase in alleviating or preventing phytate-associated, iron-deficiency anemia in humans has not been fully determined. Our objective was to determine the effectiveness of two new phytases developed in our own laboratory in releasing phytate-bound iron in corn, soy, and wheat in vivo and in vitro. Two animal experiments were conducted with 32 anemic weanling pigs fed high phytate (1.2–1.3%), corn-soy basal diet alone or the diet plus 50–70 mg iron/kg (ferrous sulfate) or different sources of phytase (1,200 units/kg) for 4–5 wk. In both experiments, dietary phytase significantly increased hemoglobin concentrations and packed cell volumes over the negative controls, resulting in status of these measures similar to that of the iron-supplemented group. In vitro, three different phytases with various levels of citric acid (0 to 6.25 g/kg) were used to maximize phytate breakdown in whole-wheat flour during bread-making and to improve dialyzability of intrinsic and added iron in the bread. Supplemental phytase (285 units/kg) plus 3 to 6 g citric acid/kg enhanced phytate reduction of the bread up to 85% and improved total iron dialyzability by up to 15-fold over the untreated bread. In conclusion, phytase may be used to effectively improve utilization of iron in plant foods for hemoglobin synthesis and to reduce human anemia incidences (Supported by a Cornell University Biotechnology grant).

234B.—Iron and Zinc Absorption in 1-yr-old Peruvian Children Consuming a Diet Based on Wheat Flour Fortified with Iron with/without Zinc and/or Vitamin A. Bo Lönnerdal,* Nelly Zavaleta[+] and Steven Abrams.** *Dept. of Nutrition, Univ. of California, Davis, CA, [+]Instituto de Investigacion Nutricional, Lima, Peru, **Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.

We measured Fe and Zn absorption in a cohort of 1-yr-old children (N = 54) who had been randomized for 6 months to receive a daily weaning meal based on milk and wheat flour fortified with Fe with/without Zn, and/or vitamin A. 58Fe-sulfate and 70Zn-sulfate were mixed with porridge (gruel) and 57Fe was given apart in orange juice with ascorbic acid. 67Zn was given intravenously. We found lower Zn absorption in Zn supplemented children, 28.1% ± 8.9 vs 37.3 ± 14.5, $p = 0.01$. Fe absorption was significantly greater in children receiving vitamin A, (57Fe: 30.1% ± 17.4 vs 19.5 ± 11.7, $p = 0.008$, 58Fe: 10.4% ± 5.9 vs 7.6 ± 3.9, $p = 0.04$). Fe absorption (57Fe) decreased by zinc supplementation when Fe + Zn was compared to Fe alone (25. 9% ± 16.8 vs 15.9 ± 8.7, $p = 0.05$). However, combined Fe, Zn and vitamin A supplementation showed comparable absorption (57Fe: 25.5% ± 12.3) as Fe alone (57Fe, 21.7% ± 12.9, $p = 0.44$). These data indicate that 6 mo of Zn supplementation leads to decreased fractional Zn absorption likely related to improved Zn status. Vitamin A enhances Fe absorption and co-supplementation of Fe with Zn and vitamin A leads to similar Fe absorption as providing Fe alone.

235B.—Incorporation of Absorbed Isotopically-enriched Iron into Erythrocytes in Adult Volunteers. Beatriz Sarria, Jack R. Dainty, Thomas E. Fox, John Eagles, Jurian Hoogewerff and Susan J. Fairweather-Tait. Institute of Food Research, Norwich, UK.

Much of the knowledge about factors affecting iron absorption in adults has been obtained from measurement of erythrocyte incorporation of radioiron (Hallberg, 1981). In normal and iron-deficient adults, Larsen and Milman (1975) used whole body counting to demonstrate that 14 days after ingestion of a radioisotope, 80–100% of the retained isotope is present in erythrocytes. Current methodology permits the determination of erythrocyte incorporation of iron with the use of stable rather than radioisotopes of iron, and studies of erythrocyte incorporation of a stable iron isotope have been conducted in adults (Barrett et al. 1992). It is assumed that 80% of absorbed iron is incorporated into erythrocytes, but the incorporation is known to vary widely in individuals (Barrett et al. 1992). By examining the enrichment of erythrocytes after the administration of an IV dose, it is possible to accurately calculate the percent incorporation of iron. On day 1 of the study, female volunteers, aged between 20 and 45 years, were given 5 mg of 57Fe as ferrous sulfate with ascorbic acid (1:3 molar ratio) in an oral dose and 200 μg of 58Fe (ferrous citrate) intravenously over 90 min. From day 11 to 27, blood samples were taken. Sample preparation involved microwave digestion and iron was extracted in all samples using ether (Roe & Fairweather-Tait 1999). Enrichment of oral and intravenous iron stable isotopes was measured in whole blood samples using a multicollector ICP-MS. Data will be presented showing how isotope enrichment varies with time.

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3. Larsen L & Milman N. (1975) Acta Med Scand 198, 271–274.
4. Roe M & Fairweather-Tait SJ. (1999) Final Project Report, MAFF Project ANO541, March.

236B.—Calculation of Iron Absorption from an Oral Dose of 57Fe Using Mathematical Modelling. Beatriz Sarria, Jack R. Dainty, Thomas E. Fox, John Eagles, Jurian Hoogewerff and Susan J. Fairweather-Tait. Institute of Food Research, Norwich, UK.

There are several methods for estimating iron absorption using stable isotope labels, including haemoglobin incorporation and plasma appearance (Kastenmayer et al., 1994; Barrett et al., 1992). An alternative approach would be to model the appearance of iron in the plasma using a single compartment model. The traditional dual stable isotope method was

compared against the proposed new method. Twelve healthy, female volunteers, aged between 20–45 years were recruited to the study. After an overnight fast, each volunteer received 5 mg of 57Fe as ferrous sulfate with ascorbic acid (1:3 molar ratio) orally and 200 μg of 58Fe (ferrous citrate) infused intravenously over 90 minutes. Blood samples were taken at regular intervals for up to 6 hours and plasma was separated. After processing, which involved HNO3 digestion, muffle ashing and extracting iron with ether (Roe & Fairweather-Tait 1999), samples were measured for isotopic abundance using an MC-ICP-MS. Labeled oral and IV plasma concentrations were calculated and area under the curve (AUC) estimations were carried out for each label and then dose corrected, to yield an estimate for iron absorption. Preliminary results show that there is a good agreement between both methods.

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2. Kastenmayer P, Davidssson L., Galan P, Cherouvrier F, Hecberg S & Hurrell RF. (1994) British Journal of Nutrition 71, 411–424.
3. Roe M & Fairweather-Tait SJ. (1999) Final Project Report, MAFF Project ANO541, March.

237B.—Iron Metabolism in Suckling and Weanling Rats Fed a Powder or In-bottle-sterilized Infant Formula. Beatriz Sarria and Maria del Pilar Vaquero. Instituto de Nutricion y Bromatologia (CSIC-UCM) Ciudad Universitaria, 28040 Madrid, Spain.

Heating involved in the manufacture of infant formulas induce the formation of Maillard reaction products (MRP), and lactulose through the isomerization of lactose. We have studied the effects of thermal processing on iron bioavailabilty of an infant formula in rats at two different stages: lactation (experiment 1) and weanling (experiment 2) and investigated the nutritional consequences on iron metabolism. A powder (PIF), previously reconstituted, and an in-bottle-sterilized liquid infant formula (LIF), from the same manufacturer were fed to suckling rats for 7 days. The same formulas were complemented with AIN-76 and fed to weanling rats for one week, after 4-day adaptation. In both experiments intake (I), body weight and the faecal and urinary excretion were monitored and the parameters: apparent absorption (A) and retention (R), and ratios: %A/I, %R/A and %R/I were calculated for iron. Hematological parameters were determined, as well as the iron concentration of liver, spleen, skin and erthrocytes. Heat markers: furosine, an indicator of MRP, and lactulose were measured in both infant formulas. 1) Food intake ($p = 0.045$) and body weight on day 7 ($p < 0.001$) were lower in LIF compared to PIF. A, R ($p < 0.05$), %A/I, and %R/I ($p < 0.001$) were significantly lower in rats fed LIF. Similarly, the %R/A was lower ($p < 0.001$) in this group. Hematocrit and hemoglobin did not show significant differences. Iron levels in liver, spleen and erythrocytes were similar in both groups, but skin iron concentration was higher in LIF ($p < 0.001$). 2) Food intake and body weight were slightly lower in LIF vs. PIF. There were no significant differences between both groups as far as metabolic or hematological parameters, neither iron tissue contents. Suckling rats fed LIF showed low food intake and therefore reduced body weight and lower iron bioavailability, but higher content of iron in skin. Results are partially attributed to the higher content of lactulose and MRP in LIF. However, these differences were not obtained in weanling rats when the proportion of LIF was reduced.

238B.—Iron Deposition in Skin of Patients with Haemochromatosis. João N. Silva,* Paulo Filipe,* Luís Cerqueira[+] and Teresa Pinheiro.[+] *Departamento de Dermatologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal and [+]Instituto Tecnológico e Nuclear, Sacavém, Portugal.

Haemochromatosis is an inherited autosomal recessive disease of iron metabolism which upon early detection and treatment, can be a manageable chronic disease, if undetected, is potentially fatal. Damage to organs is due to excessive intestinal iron uptake, which is transported to liver, heart, skin, and endocrine organs, causing skin pigmentation, development of cirrhosis, heart failure, etc.. A mutation of a gene encoding a protein that associates with transferrin receptors preventing iron-transferrin complex to enter cells, can partially explain tissue iron overload but increased gastrointestinal absorption. This work reports on preliminary data for iron deposition in skin cells in a group of patients with hereditary haemochromatosis. Skin biopsies were obtained from patients and from healthy donors. Iron depth profiles, were carried out using nuclear microscopy techniques (Nuclear Micro-probe). Distribution maps for iron were obtained, and intra- and extra-cellular iron concentration determined. Results were cross-examined with morphologic features and other essential (minor and trace) elements distribution. Skin iron content is much increased in patients with haemochromatosis when compared with healthy subjects. Extensive iron deposits are observed at granular layer and basal layer of epidermis. At basal layer iron seems to deposit preferentially at cell boundaries (5000–11000 mg/kg of iron versus 400–600 mg/kg intra-cell) as can be inferred from Fig. 1.

239B.—Influence of Organic Acids on Iron Bioavailability from Rye Bread using an In Vitro Digestion/Caco-2 Cell Model. Stine B. Sørensen,* Marianne Hansen,* Brittmarie Sandström* and Klaus Bukhave.[+] *Research Department of Human Nutrition/LMC Center for Advanced Food Studies, The Royal Veterinary and Agricultural University, DK-1958 Frederiksberg C, Denmark. [+]BioCentrum-DTU, Section for Biochemistry and Nutrition, The Technical University of Denmark, DK-2800 Lyngby, Denmark.

Introduction: Rye bread is rich in phytate, which binds iron in insoluble complexes at physiological pH and thereby lowers iron bioavailability. Addition of fermented vegetables to a high phytate meal has shown to improve iron absorption in humans, which may be due to a high content of organic acids. The aim of this study was to investigate the effect of different organic acids on iron absorption from rye bread in an in vitro digestion/Caco-2 cell model. The results could assist in selection of lactobacillus cultures with a positive effect on iron bioavailability from phytate rich meals. Materials and methods: Freeze dried, homogenized rye bread with 1.09, 2.05 and 2.85 μg phytate/g DM was suspended in water (100 mg/mL) and extrinsically labeled with 55Fe (75 kBq/mL) before 200 mM lactic acid or 100 mM acetic acid was added. The mixtures were treated with pepsin at pH 2 for 1 h at 37°C followed by treatment with pancreatin and bile at pH 7 for 1 h at 37°C. Samples were tested in Caco-2 cells cultured in monolayers on Transwell membranes (23 days post seeding, passage 33–35) after pH adjustment to 5. Cells were incubated with samples ($n = 6$) for 1 h at 37°C and subsequently harvested for liquid scintillation counting. Absorbed iron was normalized to DNA. Phytate was measured by HPIC. Results: Iron uptake was significantly reduced with increasing phytate concentration. Pepsin samples reduced iron

uptake by 24% (2.05 μg phytate/g) and 75% (2.85 μg phytate/g) compared to rye bread with 1.09 μg phytate/g. Pancreatin samples reduced iron uptake by 17% (2.05 μg phytate/g) and 56% (2.85 μg phytate/g) compared to rye bread with 1.09 μg phytate/g. Addition of 200 mM lactic acid to rye bread containing 2.85 μg phytate/g increased iron absorption in pepsin samples by 36% and 100 mM acetic acid increased iron absorption by 53% in pepsin samples. Conclusions: The Caco-2 cell model showed a dose-dependent relationship between iron uptake and phytate content in rye bread. This result is in accordance with previous human studies, which indicate that the cell model is valid for studying iron absorption in carbohydrate rich foods. Lactic acid and acetic acid increased iron absorption from rye bread in the Caco-2 cell model, indicating that this model may be applicable for further studies of the influence of different organic acids and organic acid producing lactobacillus cultures on iron absorption.

240B.—Bioavailability of Elemental Iron Powders Used for Food Fortification. James H. Swain and Janet R. Hunt. USDA-ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND, 58202, USA.

Introduction: There is little or no verification of the nutritional efficacy of elemental iron (Fe) powders used widely as food fortificants today. We determined the bioavailability of six commercially-produced (~2001) elemental Fe powders, collected for research by SUSTAIN (1). Methods: The relative biological value (RBV) of the Fe powders was measured using the AOAC hemoglobin repletion/slope ratio method (2) in 220 weanling, male Sprague-Dawley rats. Following dietary Fe depletion (24 d; ~1.5 mg Fe/kg AIN93G diet), the rats' hemoglobin was measured before and after Fe repletion with a diet (14 d; AIN93G diet) fortified with one of the six elemental Fe powders (each ~12, 24, and 36 mg Fe/kg diet), ferrous sulfate ($FeSO_4 \cdot H_2O$; ~6, 12, 18, and 24 mg Fe/kg), or no added Fe (~1.5 mg Fe/kg); n = 9–10/diet. Results: Although Fe intake and bioavailability influenced both food intake and weight gain, the relative bioavailability was similar whether based on dietary Fe (mg/kg) (see table) or absolute Fe intake (μg/d) (data not shown). Fe bioavailability of the six commercial elemental Fe powders was 21 to 64% that of $FeSO_4$, and the Fe powders differed significantly ($p < 0.05$) from each other. Carbonyl Fe powder was three times more bioavailable than the less expensive CO-reduced and reduced Fe powders. Discussion: The present findings, together with results in humans and data on commercial pricing of Fe powders, can assist in developing quantitative recommendations for fortification of foods with specific forms of Fe.

Method Fe Produced	Carbonyl	Electrolytic	Electrolytic	H-reduced	Reduced	CO-reduced
Product Name or Grade (Country) RBV1	Ferronyl (U.S.) 0.64 a (0.62–0.67)	A-131 (U.S.) 0.54 b (0.50–0.58)	Electrolytic Fe (India) 0.46bc (0.43–0.50)	AC-325 (U.S.) 0.42c (0.37–0.46)	ATOMET 95SP Canada 0.24 d (0.20–0.28)	RSI-325 Sweden 0.21 d (0.17–0.25)

1 Bioavailability (RBV; 95% C.I.) relative to $FeSO_4 \cdot H_2O$ (= 1.00); values in a row with the same letters are not different ($p < 0.05$).

1. SUSTAIN (Sharing U.S. Technology to Aid in the Improvement of Nutrition) & Micronutrient Initiative (2001) "Guidelines for Iron Fortification of Cereal Food Staples." www.micronutrient.org/frame_HTML/resource_text/publications/fe_guide.pdf.
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241B.—Distribution of Lead and Essential Minerals in Milk Fractions of Brazilian Women with Low Calcium Intake and Low Environmental Lead Exposure. Anastacio, A.S., Porto da Silveira, C.L.,* Miekeley, N.[+] and Donangelo, C.M.* *Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, and [+]Departamento de Química, Pontifícia Universidade Católica, Rio de Janeiro, Brazil.

Human milk provides adequate amounts of essential minerals with high bioavailability to the infant but it is also an infant source of lead, a neurotoxic heavy metal. In contrast to essential minerals, the pattern of lead distribution in human milk fractions is not known. The purpose of this study was to determine the distribution of lead and essential minerals in major fractions (fat, casein and whey) of mature milk of Brazilian lactating women (n = 38; 20–108 days post-partum) with low calcium intake (~600 mg/day) and low environmental lead exposure (median blood lead: 6.2 mg/dL). Milk fractions were obtained by centrifugation and ultracentrifugation of fresh milk samples. Minerals were analyzed in whole milk and milk fractions by ICP-AES (Ca, Zn) and by ICP-MS (Fe, Cu, Pb) with the following results (Mean ± SE):

	Pb	Ca	Zn	Fe	Cu
Concentration in whole milk (mg/L) (for lead: median and range, mg/L) nd = non detectable	1.3 (nd-11.9)	397 ± 7	1.8 ± 0.2	0.41 ± 0.02	0.38 ± 0. 02
Distribution in milk fractions					
Fat (%)	26 ± 5	17 ± 2	17 ± 2	17 ± 2	17 ± 2
Casein (%)	9 ± 3	7 ± 1	13 ± 2	9 ± 2	16 ± 2
Whey (%)	65 ± 6	76 ± 2	70 ± 3	74 ± 2	67 ± 3

Significant positive associations were observed between lead in milk whey and lead in maternal blood ($r = 0.53$, $p = 0.02$), and between lead and calcium in whole milk ($r = 0.52$, $p < 0.001$). Maternal blood lead was negatively associated with maternal dietary calcium ($r = -0.34$, $p = 0.02$). Our results indicate that similarly to calcium and other essential trace minerals, lead in human milk is mainly present in the whey fraction and it is possibly highly available to the infant. Increasing maternal calcium intake during lactation may limit lead transfer into breastmilk. (Financial support: CNPq, FAPERJ/Brazil).

242B.—The Effect of NaFeEDTA on Preventing Lead Poisoning in Rats. Junquan Gao, Xiaowei Li, Jun Wang and Jingling Zhao. Institute of Nutrition and Food Hygiene Chinese Academy of Preventive Medicine, Beijing 100050, China.

In this study, the effects of ferrous sulfate (FeSO4), ferrous lactate (C6H10FeO6), and sodium iron ethylenediaminetetraacetate (NaFeEDTA) on lead exclusion from the blood of Wistar rats had been compared, the rats were fed the base feed added three iron fortifiers respectively, the Fe level was 3.34 mg/kg bw in above three groups, but high NaFeEDTA group was fed 6.69 mg Fe/kg bw and were drunk freely 400 mg lead acetate /L solution for four weeks. We found that among the three kinds of iron fortifiers, Using NaFeEDTA to reduce the blood lead was the best. The lead level of blood in supplementing FeSO4, C6H10FeO6, NaFeEDTA, and High- NaFeEDTA groups were reduced 27.0 ± 10.9, 24.0 ± 12.2, 39.0 ± 19.4, and 65.7 ± 7.4 g Pb/L respectively. Base on the pilot study, the NaFeEDTA on the lead excretion effect from different organs and blood were studied and it was found out that three NaFeEDTA groups can reduce the lead levels in blood, liver, kidney and tibia, the lead level in these organs and blood in low, middle and high NaFeEDTA groups compared with lead control group were reduced significant ($P < 0.05$). There were significant reverse correlations between NaFeEDTA supplement and the lead levels in liver, kidney and tibia, and the correlation coefficients were -0.4432, -0.6134 and -0.3878 respectively.

245B.—Large Scale Purification of Selenoprotein W. A.T. Bauman* S.R. Anderson,+ D.F. Barofsky, D.A. Malencik** and P.D. Whanger.*** *Oregon State University Department of Environmental and Molecular Toxicology, +Department of Biochemistry and Biophysics, and **Department of Chemistry. Corvallis, OR 97331.

Selenoprotein W (SeW) is a major selenium containing protein found primarily in cardiac and skeletal muscle. Even though depletion of SeW is associated with white muscle disease, its function is unknown. To assist in the determination of the function of SeW, milligram quantities of the protein must be purified for use in biophysical and functional studies. Purification strategies have been developed for both native and recombinant SeW. A successful purification strategy has been developed for rat mutant selenoprotein W (RMSW, SeCys Cys, 6X N-terminal Histidine Tag), from BL21 cells. The DNA is precipitated with streptomycin sulfate from cell lysate, then clarified by centrifugation. The clarified lysate is precipitated with 40% acetone (−20°C) and the supernatant collected. Acetone is then added to the supernatant to a final concentration of 70% (−20°C). The precipitate is resuspended in phosphate buffer, applied to a DEAE cellulose column, and the flow through collected. The flow through is applied to a Ni-NTA agarose column, and eluted with imidazole. In addition, the partial purification of native SeW from rabbit has been achieved. Rabbit muscle extract (~.6 mg SeW/L) is adjusted to pH 6.0 and the supernatant collected. The supernatant is heated to 60°C for 3 minutes and cooled to 4°C. The supernatant is applied to a CM cellulose column, which is eluted at 500 mM NaCl. Additional purification steps are in development.

247B.—Selenium and Metallothioneins in Liver of Hens Fed with Sunflower and Linseed Oil, respectively. Ingrid Falnoga, Vekoslava Stibilj, Robert Vindiš, Majda Tušek-Žnidarič, Antonija Holcman* and Janez Šančar. Jožef Stefan Institute, Ljubljana, Slovenia and *Biotechnical Faculty, Ljubljana, Slovenia.

High fat diet can generate increased amounts of lipid peroxides and consequently the changes in selenium and metallothioneins levels. The metabolism of selenium, its affinity to metallothionein (MT) and its distribution in the liver tissue of laying hens (Isa Brown Red) exposed to monosaturated or polyunsaturated fats was investigated. The experiment was performed with three groups of six hens fed for 42 d with either a standard diet containing 0.20 mgSe/kg or with the same diet enriched with 7% of extra sunflower oil and 7% of linseed oil, respectively. Extra sunflower oil contained about 80% oleic and 12% linoleic (omega-6) acid, and linseed oil contained about 20% oleic, 14% linoleic and 58% alfa-linolenic (omega-3) acid. In both groups Se liver concentrations increased regarding the control group. Increased levels were found in lyophilized tissue samples, in water-soluble extract and in different Se peaks after size exclusion chromatography of extracts on Sephadex G-75. Slightly higher increase was found in-group fed with sunflower oil. Beside increased selenium adoption we expected the induction of metallothioneins as a consequence of fat stress. But the results did not show any significant difference between the groups except the increase of Se associated with Zn, Cu-thioneins in the groups fed with high fat diet.

248B.—In Vitro Evaluation of Selenium Compounds as Antioxidants Against Reactive Oxygen and Sulfur Species. Niroshini M. Giles, Karen M. Tasker, Gregory I. Giles and Claus Jacob. School of Chemistry, University of Exeter, Stocker Road, Exeter, Devon, United Kingdom.

Oxidative stress is a biochemical condition present in several human disorders, characterised by a change in the cellular redox state due to the production of reactive oxygen (ROS) and reactive sulfur (RSS) species. Cellular antioxidant defense systems utilize the selenium containing enzyme glutathione peroxidase (GPx) to detoxify these oxidants, in the process consuming the cellular redox-buffer glutathione (GSH). GPx can be synthetically modeled to produce organoselenium drugs that mimic its catalytic antioxidant activity. To further develop this concept a range of novel selenium antioxidants have been synthesized and evaluated in vitro according to their ability to detoxify species known to be present under oxidative stress conditions. At high concentrations of GSH the compounds demonstrate GPx activity. Under highly oxidising conditions cellular levels of GSH are depleted with a corresponding increase in RSS. It is therefore essential to examine potential antioxidant therapeutics against these drug targets. The catalytic destruction of tert-butyl hydrogen peroxide (ROS), and cystamine-S-monoxide (RSS) was readily accomplished by incubation with selected GPx mimics and the progress of the reactions monitored by UV-Vis spectroscopy. From this data structure-activity relationships were obtained and referenced to known organoselenium compound such as ebselen. The accumulation of this in vitro data in addition to the information already present from compounds in the Exeter Antioxidant Depository provides a sufficient pool to allow the prediction of structural features that will greatly assist future drug design. Knowledge of the chemotypes required for antioxidant activity will either enable drugs to possess a broad spectrum of activity or alternatively to be specifically targeted against one class of oxidizing species.

249B.—Evaluation of Selenium Contents in Diets of Preschool Children from the Amazon Region in Brazil. Irland Barroncas Gonzaga,* Vera Akiko Maihara[+] and Silvia M. Franciscato Cozzolino.[±] *Universidade Federal do Pará, Belém-PA, Brazil, [+]Instituto de Pesquisas Energéticas e Nucleares, LAN-CRPa, São Paulo-SP, Brazil and [±]Universidade de São Paulo, São Paulo-SP, Brazil.

Aim of the current work was the evaluation of the selenium contents in diets of 129 pre-school children of both sex (3–6 years old). The diet was controlled during a period of 7 month (minimum) up to 2 years and on five days the week. The children received breakfast, lunch, snack and dinner (4 meals per day) composed from typical, exotic foods of the Amazon region. The components were of origin from Pará and Amapá. Included were manioc flour, tapioca and cake of macaxeira (both products of manioc), açaí (a type of palm fruit), river fishes, acerola (a fruit), Brazil nuts and more. The meals of 7 days were collected separately and the fresh weight was determined. After consequent lyophylisation the samples were analyzed in an interlaboratory collaboration by neutron activation analyses (NAA) and hydride generation atomic absorption spectrometry (HG-AAS). The dates of the two applied analytical techniques do not show large statistical differences. In average a daily dietary intake of 37,4 μg/day selenium was determined in Pará, whereas in Amapá an average of 106,5 μg/day was observed. The daily selenium intake of the Amapá children group differ significantly from the recommended values. The recommendation of the NAS, 2000 for children of this age (3–6 years) was exceeded approximately 2 times in Pará and 5 times in Amapá by the average of the determined selenium levels. Symptoms of an selenium intoxication were not observed. The remarkable difference is mainly caused by the presence of Brazil nuts in some of the diets from Amapá. On days with nuts supplements, the daily selenium intake increased up to 279,3 μg/day, which is approximately 14 times higher than the recommended values (RDA). Selenium levels in a representative diet of a low income group of adult persons from the Amazon region ranged between 87.3–107.7 μg/day, which is as well above the recommended diary allowance (RDA) for selenium. The results reveal surprisingly high selenium concentrations in the children diet of the Amazon region and further investigation are advised to avoid negative effects of an undesired overdoses. Special attention should be paid to the role of Castanha-do-Pará (Brazil nuts) within the diet. Brazil nuts contain the highest selenium levels, which are up to now known from a fruit used in the human alimentation.

National Academy of Science (NAS), 2000; Amaroux et. al, 2001; *Favaro, D. et al, 1997, World Health Organization (WHO), 1996; E. O. Uthus, C.D, Seaborn, J. Nutr.; 126 (1996) 2452S. Financially supported by CAPE.

251B.—Selenium Deficient Status of East Bohemia Seniors and its Improvement During Two Years of Selenium Supplementation. Jan V. Kvicala,* Jana Vrbikova,* Vaclav Zamrazil* and Vaclav Jiranek.[+] *Institute of Endocrinology, Praha, Czech Republic and [+]DataPro, Praha, Czech Republic.

Selenium is an essential trace element that in form of selenoproteins has many protective and regulatory functions necessary for animals and man. Decrease of selenium status in seniors has been repeatedly reported especially in the countries with less or more profound selenium deficiency. Increase of some diseases like malignity, cardiovascular diseases, neural or inflammatory diseases is known in old age as well. Rather low concentrations of selenium in serum (56 ug/L) and in urine (8.8 ug/L, 12.4 ug Se/g creatinine, respectively) were determined in 108 seniors of East Bohemia, with a slight but significant correlation between these indexes of selenium status. Supplementation by sodium selenite started with the 50 ug Se/day for the first 3 months, to be increased to 100 ug Se/day after that time. Increase of serum and urine selenium was significant already after 7 weeks (84 ug/L, 18 ug/L, respective) both in comparison with the placebo group and with the initial status. The course of the serum Se concentration curve showed the first peak in 19 weeks (97 ug/L). Small decrease of serum Se concentration (90 ug/L) appeared on 52nd week in spite of increased amounts of supplemented Se. Slow increase followed till the 104th week (110 ug/L). Sharp decrease to 73 ug Se/L serum developed during 7 weeks after the end of the supplementation. Urine selenium curve was similar, with the maximum on 26th week (34 ug/L) and even sharper decrease after the end of supplementation (15 ug/L—no significant difference with placebo group). The study was partly supported by grant IGA MZ CR No. 5392-4.

252B.—Increase of Selenium Status of Inhabitants of Jindrichohradecko, South Bohemia. Jan V. Kvicala,* Vaclav Zamrazil* and Vaclav Jiranek.[+] *Institute of Endocrinology, Praha, Czech Republic and [+]DataPro, Praha, Czech Republic.

Essential role of Se has been well established. Its deficiency may afflict several regulatory and protective functions of selenoenzymes like the regulation of the metabolism of thyroid hormones, antioxidative protection, control of synthesis of prostaglandines, prostacyclines, tromboxanes and leucotrienes, detoxification of heavy metals and carcinogenic organic compounds. Selenium deficiency may decrease immune functions, reproductive functions, as well as cognitive functions and increase senility. Very low status of Se has been revealed in inhabitants of region Jindrichohradecko seven years ago. Mean serum Se concentration of inhabitants between 10 and 65 years was 48,6 ug/L—less then one half of optimal values. Urine Se has mean level 7.6 ug Se/L urine, 6.7 ug Se/g creatinine, resp.—very low in comparison with the countries with adequate Se intake (40–100 ug Se/L urine). Mean Se intake assess from urine Se concentration was 15–23 ug Se/day, which level is near the minimal level for ensuring life necessary functions of the selenoproteins. M.P. Rayman published decrease of Se intake in the West Europe. In opposite, there were some signs of increase of Se in the food chain of some regions in the Czech Republic. That is why new study of Se status was started in this region in the year 2001. Increased levels of the serum and urine Se have been found in the region—mean serum Se was 56.8 ug/L and mean urine Se was 16.8 ug/L, 13.0 ug Se/g creatinine, resp., which means the increase of Se intake to 33–50 ug/day. Increase of body Se has been proved for all age and gender groups, with the highest increase for girls. The work was supported by grant IGA MZ CR No. 5392-4.

253B.—Effects of Selenium Deficiency on Ascorbic Acid and Dehydroascorbic Acid Levels and Oxidation Status in Guinea Pigs. Mary R. L'Abbé, Keith D. Trick, Alexandre Giroux, Nick Hidiroglou, Réné Madère, Robert Peace and Penny Jee. Nutrition Research Division, Health Products and Food Branch, Health Canada, Ottawa ON, Canada K1A 0L2.

Guinea pigs, like humans, are unable to synthesize their own vitamin C and are reliant on an external source of vitamin C in their diet. Vitamin C in its reduced form (ascorbic acid, AA) is a vital component of many biological processes, particularly as a non-enzymatic antioxidant. The selenium-dependent enzyme, thioredoxin reductase (ThR) can reduce many substrates, including the oxidized form of vitamin C (dehydroascorbic acid, DHAA) to the reduced ascorbate (AA) form. The aim of this study was to investigate the role of selenium and selenium deficiency in vitamin C metabolism. Weanling male Hartley inbred guinea pigs (Elm Hill Breeding Labs), approximately 10 days of age, (mean body weight 156 g) were randomly assigned by body weight into 4 groups of 11 animals each. Guinea pigs were fed a vitamin C-deficient, torula yeast based diet containing varying amounts of selenium (0, 50 or 200 μg Se/kg diet). Ascorbic acid solutions were prepared fresh and animals were orally dosed with 0.3 mg (marginal) or 2.4 mg AA/100g body wt/day (normal) respectively. Treatment groups were: Group 1, Se deficient, marginal C; Group 2, marginal Se, marginal C; Group 3, normal Se, marginal C; and Group 4, normal Se, normal C. After 5 weeks, erythrocyte glutathione peroxidase activity was reduced to 51%, 56%, and 71% of the control (Group 4) levels. Total vitamin C levels in liver were 13.1 $\pm$ 6.5, 16.3 $\pm$ 5.9, 15.3 $\pm$ 4.3 and 39.1 $\pm$ 10.9 μg/g in Groups 1–4 respectively ($p < 0.0001$). The proportion of total vitamin C as AA decreased and DHAA increased resulting in DHAA/AA ratios of 2.5, 1.4, 2.1 and 0.8 respectively, which was increased with marginal C compared to normal C, but did not differ significantly between animals fed differing amounts of Se. Plasma levels of reduced GSH were unchanged with differing amounts of Se (7.5 $\pm$ 1.2, 8.1 $\pm$ 2.1, 7.0 $\pm$ 1.5 μM/L) but were significantly lower than in the normal vitamin C group (45.3 $\pm$ 14.4). Thus, Se deficiency did not cause further oxidation in guinea pigs fed marginal vitamin C.

254B.—Further Identification of Selenoprotein N, a Protein of Unknown Function Responsible for a Congenital Muscular Dystrophy. Alain Lescure,* Nathalie Petit,[+] **Behzad Moghadaszadeh,**[+] **Ulla M. Wewer,** Alain Krol* and Pascale Guicheney.**[+] *UPR 9002 du CNRS, Institut de Biologie Moleculaire et Cellulaire, Strasbourg (France); [+]INSERM U523, Institut de Myologie, Paris (France), **Institute of Molecular Pathology, University of Copenhagen (Denmark).

The gene SEPN1 encodes SelN, one of the new selenoproteins identified by a computer-assisted approach (Lescure et al., 1999). SEPN1 is located on chromosome 1 p35–36 and comprises 13 exons. At the transcript level, two variants have been identified, a full length coding for a protein of 590 amino acids and a shorter variant in which exon 3 consisting in an Alu-cassette is spliced-out (Moghadaszadeh et al., 2001). The cDNA harbors a characteristic SECIS element within the 3′ UTR, which is mandatory for selenocysteine insertion into the protein, and two potential selenocysteine UGA codons: one in exon 3 at position 127 and a second one in exon 10 at position 462 of the protein. Examination of the SelN sequence revealed no homology to any known protein. The only conserved motif is a calcium binding site located in the N-terminal part of the protein. The function of the protein remains elusive so far. Query of nucleotide databases identified orthologs of SelN in different vertebrates (mouse, rat, bovine, chicken or zebrafish), but no homologous sequences could be detected in invertebrates (drosophila) or lower eukaryotes (C. elegans, yeast). Northern blots showed that the SelN mRNA is ubiquitously expressed, with a higher expression pattern in heart and pancreas. The SEPN1 gene was identified as a positional candidate gene for Rigid Spine Muscular Dystrophy (RSMD1) in patients with a rare autosomal recessive neuromuscular disorder characterized by early rigidity of the spine, axial and proximal muscle weakness, limb-joint contractures and respiratory insufficiency. (Moghadaszadeh et al., 2001). Several mutations, including frameshift, missense or nonsense mutations have been identified in the coding sequence. Further analysis confirmed the absence of the SelN protein in cultured skin fibroblasts from two patients carrying homozygous frameshift mutations. Identification of the SelN function should provide a better understanding of the pathophysiological mechanisms leading to the muscle pathology.

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255B.—Evaluation of Nutritional Status of Selenium in Elderly Women in São Paulo, Brazil. Vanuska Lima da Silva,* Vera Akiko Maihara,[+] Mitiko Saiki,[+] Luiz Cláudio Silva* and Silvia M. Franciscato Cozzolino.* *Universidade de São Paulo, São Paulo-SP, Brazil and [+]Instituto de Pesquisas Energéticas e Nucleares, LAN-CRPa, São Paulo-SP, Brazil.

The aim of this study was to evaluate the Selenium nutritional status in non institutionalised elderly women. Thirty women were studied with age >60 years that were not taking any vitamin-mineral supplements nor presenting any pathology that could compromise the study, such as cancer, diabetes or rheumatoid arthritis. Replicate samples of the food had by each of the participants were collected for analysis of the chemical composition and for Selenium determination by neutron activation analyses (NAA). The average of the daily ingestion of Selenium was measured as 47,2 mg/day, despite of 80% of the participants being below the EAR (Estimated Average Requirement), which is 45mg/day for the investigated age. Blood samples were as well collected to determine concentration of Selenium in both plasma and erythrocyte, the latter by Fluorimetric method. The average of Selenium concentration in plasma and in erythrocyte were 79,8 mg/L and 81,4 mg/L, respectively. Although there is no well defined pattern for the establishment of the Selenium nutritional status, it can be considered, from the obtained results, that the studied group was deficient in Selenium, similarly to what was found for other groups of different ages studied in other regions of Brazil.

NRC (National Research Council), 2000; LEVANDER, 1985; FOSTER, 1997; DRUCOS, 1997. Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

256B.—Mapping of Selenium Distribution in Brazil: Analyses of Natural Pathways from Soil to the Human Diet. Andreas Martens-von Salzen,* Irland Barroncas Gonzaga[+] and Silvia M. Franciscato Cozzolino.[+] *Institut für Anorganische und Analytische Chemie, Technische Universität Braunschweig, Braunschweig, Germany and [+]Dept. de Alimentos Nutrição Experimental, Faculdade de Ciências Farmaceuticas, Universidade de São Paulo, São Paulo, Brazil.

Today, selenium has been accepted as essentially for the animal and human health. The range between deficiency (<11 mg/day), necessity and toxicity (>900 mg/day)[1] is small and manifestation of several diseases is observed when the limits are passed permanently. The knowledge about the concentration in the selenium sources is a precondition for the right prevention. Selenium levels in meat, vegetables and fruits reflect selenium levels in soils on which the cattle fed or where the plants were cultivated. Its distribution in soils is determined for several countries, but little is known about Brazil.[3,4] As our contribution to fill this gap, we collected soil in several Brazilian states and analyzed the selenium contents by hydride generation atomic absorption spectroscopy (HG-AAS). A direct relation between selenium levels in soil and in human is reported from some regions in China.[3] To examine the geographical influence onto the human selenium sources, we followed the natural pathway from the soil to the human diet and analyzed selenium in meat and beans, but also in pasture and food supplements of the cattle, as well as in bean plants, soils and water of their origin and compared it with representative diets of different social groups from several Brazilian states. The daily dietary selenium intake depends not only on the region, but differs also significantly with the social group. The selenium contents in a representative diet of a low income group from the south of Brazil ranges between 45.1–59.2 g/day, whereas in the same region the values for the diet of a high income group vary between 132.1–146.0 g per day. The social influence and the dependency from personal habits is demonstrated in the daily dietary selenium intake of a students group, which is remarkable low and ranges between 30.2–34.7g/day. Different nutritional habits from the south are found in the Amazon region. The daily dietary selenium intake of a low income group from this region is slightly above the recommended dietary allowance (RDA) and vary between 87.3–101.7 g/day. In latest results of pre-school children groups from Pará and Amapá the recommended values were exceeded several times. A combined overview about our results in Brazil will be presented.

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257B.—Protective Role of Intraperitoneally Administered Vitamin C, E and Selenium on the Levels of Lipid Peroxidation in the Testes of Rats Made Diabetic with Streptozotocin. Naziroğlu, M. Department of Physiology Firat University, Veterinary Faculty, TR-23119, Elazig, Turkey.

Vitamin E is a lipid-soluble chain breaking antioxidant and is an important antioxidant in biological systems. Glutathione peroxidase, GSH-Px, is an important enzyme which need additional selenium (Se) for its activity. The primary role of vitamins E, C and GSH-Px in metabolism appears to be reduction of polyunsaturated fatty acid (PUFA) hydroperoxides and hydroperoxides to their hydroxy acid analogs, thereby preventing lipid-free radical chain reaction. Testes are particularly susceptible free radical chain reaction because the concentration of PUFA in the membranes is high. Also, abnormalities of testes are very common in diabetic subject. However, there is insufficient inform about the antioxidant enzymes levels of testes during diabetes. Therefore, I have undertaken to screen protective role of vitamin E, C and Se in the testes of diabetic rats. In the study, sixty adult male Wistar rats were used and all animals were randomly divided into six groups. The first group was used control (C) and second group as diabetic control (DC). Placebo was injected to first and second groups. The third group was intraperitoneally administered with vitamin E 20 mg/ over day), the fourth group with Se (0.3 mg over day), and fifth group with their combination. The sixth group was intraperitoneally administered with vitamin C (30 mg/over day). This administration was done for 25 days. Fourth days after the injection of vitamin E, C and Se, animals in all

groups except first group were made diabetic by intraperitoneally injection of streptozotocin (STZ). After the induction of diabetes, vitamin E, C and Se administration were also performed 21 concessive days. The lipid peroxidation (MDA), GSH-Px, reduced glutathione (GSH) levels were determined. Values were evaluated by Mann-Whitney U test of SPSS packed program. GSH-Px activity and GSH level were significantly ($P < 0.05$) higher in C group than in DC group whereas MDA levels was slightly lower. GSH levels and GSH-Px activity were significantly ($P < 0.05$-$P < 0.001$) higher in vitamins E, C and Se and combination groups than both C and DC groups whereas MDA levels in the groups were significantly ($P < 0.01$, $P < 0.001$) decreased. In conclusion, the results from these experiments indicate that vitamin E, C and Se have significant protective effects on the testes against oxidative damage, but the effects of vitamin E appears to be much greater than that of Se and vitamin E.

258B.—Selenium Requirement of Growing Pigs Determined by Different Parameters. Josef Pallauf, Othmar P. Walz, Alexandra Fischer and Anika Wagner. Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, D-35392 Giessen, Germany.

The aim of the study was to determine the selenium requirement of growing pigs based on parameters of Se status and oxidative cell damage. Over a period of 7 weeks 5x7 male weanling pigs (initial average weight: 8.76 ± 0.04 kg) were fed Se deficient diets (<30 μg Se/kg diet) based on torula yeast, wheat, soybean oil and coconut oil containing different supplements of Se. Se was added as sodium selenite in the concentrations 0 μg (group I), 50 μg (group II), 100 μg (group III), 200 μg (group IV) or 300 μg Se (group V) per kg diet. In all groups 10 IU of vitamin E were supplemented to a native a-tocopherol content of 5.5 mg/kg diet. Blood samples were taken at the beginning and end of trial (average weight: 34.3 ± 1.06 kg) and every second week. Activities of enzymes were measured in the blood and liver and expressed per mg protein (p). The dietary regime had no significant effect on feed intake and daily live weight gain (548 ± 24.9 g). Different Se supplements affected whole blood Se concentration significantly in a dose dependent manner up to 200 μg/kg diet. Similarly plasma GPx (pGPx) showed significantly increasing activities from group I (1.41 ± 0.07 mU/mg p, week 7) to group IV (4.90 ± 0.64 mU/mg p, week 7). The highest Se supplementation (group V) did not further enhance blood Se concentration and pGPx activity. Of the Se dependent enzymes evaluated, cellular glutathione peroxidase (cGPx) in the liver seemed to be the most sensitive parameter to reflect the Se status. With decreasing Se in the diet, cGPx activity fell significantly from 127 ± 17.8 mU/mg p (group V) to 12.4 ± 1.80 mU/mg p in group I, which represents a reduction in cGPx activity of 90.4%. The activity of thioredoxin reductase (TrxR) in the liver was evaluated in mU/mg p as 1.60 ± 0.28 in group I, 2.82 ± 0.36 in group II, 3.76 ± 0.34 in group III, 3.46 ± 0.19 in group IV and 4.80 ± 0.53 in group V. Furthermore, Se deficiency in group I decreased phospholipid hydroperoxide GPx (PHGPx) activity in the liver significantly compared to the other groups. Glutathione-S-transferases, which are believed to compensate in part the decrease in cGPx activity, were increased in activity in the liver of Se deficient animals in group I. Parameters of cell damage and inflammation (TBA-RS, glutamine synthetase) demonstrated marked differences between groups, whereby group I receiving no Se supplement showed severe lesions. Under the conditions investigated a minimum requirement of 200 μg Se/kg diet for growing pigs within a live weight range of 8–35 kg was necessary to maximize the activity of most Se dependent enzymes and to prevent oxidative cell damage.

259B.—Selenium Requirement of Growing Rabbits. Josef Pallauf and Susanne Blind. Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, D-35392 Giessen, Germany.

As there is no precise Se recommendation for rabbits, commercial rabbit diets vary considerably in their Se content. In this study 60 growing New Zealand White rabbits were therefore fed a Se deficient basal diet (<0.03 mg Se/kg) based on torula yeast and wheat as a feed supplementation during the suckling period. An adequate-tocopherol concentration of 30 mg/kg diet was maintained. After weaning at 4 weeks the rabbits had reached an average weight of 623 ± 78.6 g and were divided into 5 groups of 6 males and 6 females each receiving the basal diet supplemented with Se as sodium selenite in increasing concentrations (0.0 mg/kg diet group I; 0.05 mg/kg group II; 0.1 mg/kg group III; 0.15 mg/kg group IV; 0.2 mg/kg group V) for 9 weeks. Additional animals of both sexes were sacrificed to obtain the initial status of the parameters investigated. During the trial there were no significant differences between the 5 groups in daily weight gain (37.4 ± 8.1 g/d) and feed conversion efficiency. Blood was taken every two weeks from the vena auricularis. Compared to the initial Se concentration in plasma (92.8 ± 8.2 μg/L) there was a significant decrease in group I (30.8 ± 4.4) and significant increases in the remaining groups (II: 136 ± 16.5; III: 149 ± 16.5; IV: 147 ± 11.1; V: 158 ± 14.4) after 9 weeks. Between group II and V the difference was significant. Similar results were obtained for the glutathione peroxidase activity in plasma (pGPx). Group I had a significantly lower activity than the other groups (II, III, IV, V) with group V having a significantly higher activity of pGPx than group II. The activity of the cytosolic GPx (cGPx) in the liver was very low in group I (8.6 ± 1.5 mU/mg prot.) compared to group II (227 ± 54.9). Significantly higher activities could be observed in group III (454 ± 152), IV (450 ± 87.6) and V (535 ± 120) with no significant differences between these 3 groups. The measurement of cGPx activity in the thymus, an organ higher in the hierarchy of stability of enzyme activity in Se deficiency, showed different results. While the activity of group I (19.5 ± 3.2 mU/mg prot) was once more significantly lower, group II (56.3 ± 8.8), III (55.5 ± 5.8), IV (62.9 ± 7.1) and V (58.5 ± 4.0) nearly had the same activity. These results show that a Se concentration of about <0.1 mg/kg diet is sufficient to reach the maximum cGPx activity in the thymus and almost a maximum of pGPx, whereas a Se concentration of about 0.15 mg/kg and possibly more is needed to obtain a maximum of cGPx activity in the liver.

260B.—Are Dietary Allowances for Selenium Adequate for Smokers? E Paterson,* CD Thomson* and TA Mori.[+] *Dept of Human Nutr, Univ of Otago, Dunedin, NZ and [+]Dept of Med, Univ of Western Australia, Perth, Australia.

Low dietary selenium in NZ, allows assessment of selenium supplementation on oxidative stress. Smokers are under continual oxidative stress from high levels of smoke-induced free radicals and inflammation. In a randomized, double-blind study, smokers screened for low blood selenium (n = 72), were supplemented daily with selenomethionine (100 g) or placebo for 20 weeks. Blood antioxidants and urinary F2-isoprostanes were determined before and during supplementation. At baseline, compared to non-smokers (n = 30), smokers had significantly lower selenium ($P < 0.001$) and ascorbic acid levels ($P = 0.004$); moreover, heavy smokers were lower than moderate smokers ($P = 0.026$ Se plasma, $P = 0.011$ Se whole blood, $P = 0.005$ GPx whole blood, $P = 0.001$ ascorbic acid). In response to supplementation, plasma and whole blood selenium concentrations and whole blood GPx activities increased significantly ($P < 0.001$). Subgroup analysis indicated significant responses in males ($P = 0.002$) and females ($P = 0.001$), as well as in moderate ($P < 0.001$) and heavy smokers ($P < 0.001$). At weeks 5 and 15, 4-day diet records indicated that male smoker dietary selenium intakes (58g/d) were similar to those of NZ males (60 g/d); but female smokers had selenium intakes (38 g/d) slightly lower than NZ females (44 g/d)–intakes lower than the 2000 RDA (55_g/d). The 18% and 24% increase in plasma and whole blood GPx activity, respectively, in response to a selenium intake of 138–158 g/d suggests that NZ smokers are not selenium-replete. Thus, the 2000 RDA for selenium is inadequate for NZ smokers.

261B.—Selenium Status Predicts the Risk of the Pregnancy Disease Pre-eclampsia in UK Women. Margaret P. Rayman,* Peter Bode[+] and Christopher W.G. Redman.[=] *School of Biomedical and Life Sciences, University of Surrey, Guildford GU2 7XH, UK; [+]Delft University of Technology, Interfaculty Reactor Institute, Mekelweg 15, 2629JB Delft, The Netherlands; [=]Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, University of Oxford, Oxford OX2 9DU, UK.

Pre-eclampsia is a serious complication of pregnancy involving damage to the endothelium. Oxidative stress has been implicated in its pathophysiology and antioxidant vitamins have shown a beneficial effect in a high-risk pregnant group. Selenium is an antioxidant trace element and so might be expected to be able similarly to reduce the risk of pre-eclampsia. Furthermore selenoproteins can scavenge the powerful oxidising agent peroxynitrite which is formed in the placenta and vasculature of pre-eclamptic women. In this study, we investigated the possible role of selenium status in the etiology of pre-eclampsia. Toenails, laid down from 3–12 months prior to clipping, were collected from 52 pre-eclamptic patients and 52 pregnant controls, matched for age, gestation and parity, at the John Radcliffe Hospital, Oxford. Clinical characteristics of the patients were recorded. The selenium content of the toenails was determined by instrumental neutron activation analysis. Wilcoxon's Signed Rank test for paired data showed that median toenail selenium concentrations in the pre-eclamptic women (0.56, interquartile range 0.51–0.64 mg/kg) were significantly lower than in their matched controls (0.62, interquartile range 0.57–0.69 mg/kg) ($p < 0.001$). The results of this study show that in UK pregnant women, low selenium status spanning the period from 3–12 months before diagnosis, is significantly associated with increased risk of developing pre-eclampsia. Adequate selenium status may reduce the risk of developing this oxidative-stress condition through the action of the antoxidant selenoenzymes, or by the scavenging of peroxynitrite in the plasma by selenoprotein P, an ability unique to this selenoprotein.

262B.—Influence of Breed on Selenium-glutathione Peroxidase Activity in Cattle. Jason E. Rowntree, David R. Hawkins, Gretchen M. Hill, Jane E. Link, Michael J. Rincker, J. Brett Barber and Jr. Robert A. Kreft, Michigan State University, East Lansing, MI, USA.

The influence of genetics on protein expression has resulted in the observation of racial differences in humans and breeds in other mammals for many important proteins. Our lab previously reported that plasma copper concentration was influenced by beef breed and age (Ehnis et al., 1996), but little is known about the influence of breed and age on selenium (Se) dependent glutathione peroxidase (GPX1), an indicator of Se status. Michigan is a Se deficient area, hence resident livestock are prone to Se deficiency. Therefore, our objective was to determine if cattle breed and age influence GPX1 activity in the red blood cell. Angus, Hereford, and Holstein (n = 37, 44, 32) yearling heifers, two-year old dry (non-lactating) cows, and aged dry cows (three years and older) from the Michigan State University herds were bled. Red blood cells were isolated, washed and GPX1 activity was determined by the method of Paglia and Valentine (1967). All cattle were routinely supplemented Se as selenite in a trace mineral salt. Holstein females (dairy breed) had higher GPX1 activity ($P < 0.01$) than Angus and Hereford females (beef breeds) (20.97 vs 17.46 and 18.03 GPX1 EU/g Hb, respectively). Yearling females of all breeds had higher enzyme activity ($P < 0.01$) than two-year old or aged cows (21.38 vs 17.48 and 17.62 GPX1 EU/g Hb, respectively). Holstein heifers and two-year olds did not differ from Angus or Hereford heifers and two-year olds. However, Holstein cows exhibited greater GPX1 activity ($P < 0.01$) in the red blood cell than the Angus or Hereford cows (20.78 vs 16.24 and 15.83 GPX1 EU/g Hb, respectively). There were no differences between Angus and Hereford females for GPX1 activity. These results indicate that Holstein cattle have higher Se status than Angus and Hereford cattle as measured by GPX1. Furthermore, the data suggests that Holstein cows, through genetics or management, maintain higher Se levels through their production life.

263B.—The Epidemiological Study of the Se Status in Adult Humans, Living in Different Regions of Russia. A.V. Skalny and N.A. Golubkina. Center for Biotic Medicine, Moscow, Russia; Institute of Nutrition, Russian Academy of Medical Sciences, Moscow, Russia.

Today the determination of the Se concentrations in serum, and hairs are most used approaches to estimation (evaluation) of the Se status in humans. During 1992–2001 there were investigated 2462 serum and 17331 hair samples, collected in 20–50 years old inhabitants of 17 regions of Russia. The routine spectrofluorimetric (for serum) and ICP-AES (for scalp hair) analysis were provided. The range of hair and serum Se were 0.66–1.5 ppm

and 0.95–1.47 mcm/l, respectively. The obtained data revealed the suboptimal Se serum and hair concentration in Ryazan, Bryansk, Tula, Smolensk (central and western regions of European part of Russia) and lowest serum Se in Irkutsk region (Eastern Siberia). The lowest hair, but not serum Se in Vologda region (north-western region of European part) was observed. The maximal hair Se concentrations were typical for Nizhniy Novgorod (central regions of European part), Murmansk (European North) and maximal serum Se—for Novosibirsk, Krasnoyarsk (Siberia), Nizhniy Novgorod, Tver, Murmansk (central regions of European part) and perm (Ural). The statistical analysis demonstrated the strong positive correlation ($r = 0.62$) between hair and serum Se concentrations in 14 regions. So, the presented study the comparability and usefulness of both hair and serum Se determinations in epidemiological studies suggested.

264B.—Selenium Regulation Uniquely Switches Glutathione Peroxidase-1 from Decay to Translation. Roger A. Sunde. Molecular Mineral Nutrition, Nutritional Sciences and Biochemistry, University of Missouri, Columbia MO.

Selenium regulation of glutathione peroxidase-1 (GPX1) expression is novel and unique relative to the metal regulation of many markers of mineral status. In rats, Se deficiency results in >90% decreases in both GPX1 activity and mRNA, whereas glutathione peroxidase-4 (GPX4) activity and mRNA decrease 60% and <10%, respectively. To study the effect of Se status on thioredoxin reductase (TRR) activity and mRNA, we compared TRR regulation directly with other selenoproteins in male weanling rats fed Se-deficient (−Se) diets or supplemented with Se (+Se) for 28 days. TRR activity in −Se liver decreased to 15% of +Se activity versus 2% and 40% for GPX1 and GPX4, respectively. Using ribonuclease protection analysis (RPA), TRR mRNA levels in −Se liver decreased only to 70% of +Se levels. Thus TRR represents a third pattern of Se regulation with dramatic down-regulation of enzyme activity but only a modest decrease in mRNA level. The Se regulation of GPX1 expression is mediated by mRNA stability and involves nonsense-mediated decay (NMD). To evaluate the relative contribution of mRNA abundance versus translational efficiency in Se regulation of GPX1 expression, we quantitated GPX1 and GPX4 transcripts per cell in rat liver. In −Se liver, GPX1 transcripts are moderately abundant, similar to other selenoprotein mRNAs; Se supplementation increases GPX1 mRNA 8–10-fold. Translational efficiency of GPX1 mRNA is half of that of GPX4, and increases 20-fold in + Se liver. This regulatory switching of GPX1 mRNA from NMD to translation can explain why GPX1 is an excellent parameter for assessment of Se status relative to many markers of mineral status. (USDA #98-35200-6051).

265B.—Proposed Australian and New Zealand Nutrient Reference Values for Selenium and Iodine. Thomson, C.D. Department of Human Nutrition, University of Otago, Dunedin, New Zealand.

In 1998 agreement was reached between the Commonwealth Department of Health and Aged Care in Australia and the New Zealand Ministry of Health, for a joint Australia New Zealand review of nutrient reference values (NRVs), and subsequently, a framework for the review was developed. The Ministry of Health has undertaken to develop the technical review papers for key nutrients, such as selenium and iodine, and contracted the services of the author to prepare these reports. The proposed values have yet to be adopted for Australia or New Zealand. In accordance with the agreed framework, the new values have been called Nutrient Reference Values (NRVs) for Australia and New Zealand and include six reference values: Reference Nutrient Intake (RNI), Estimated Average Requirement (EAR) or Adequate Intake (AI) if EAR cannot be estimated, Critical Low Intake (CLI), Upper Safe Limit (USL), Provisional Functional Range (PFR) and Toxic Threshold (TT). The main criterion for estimating the EAR and RNI for selenium was maximization of plasma glutathione peroxidase (GPx). This is in accordance with most other committees around the world, in particular the US and Canadian Dietary Reference Intake (DRI) Committee. As for other recommendations, possible additional health effects of higher intakes of selenium were not considered in the estimation of the new RNI, because the evidence is inconclusive and there is insufficient data on which to base a numerical value for a possible protective level. However, they have been considered in the PFR. NRVs for pregnancy, lactation, infants and children have been estimated using similar criteria as for the US and Canadian DRIs. However, Australian and New Zealand data have been used when available, such as for selenium concentration in breast milk and body weights for the different age groups and gender. Similarly, Australian and New Zealand data have been used when available in the estimation of NRVs for iodine. However, in general selection indicators for estimating requirements are similar to those used by the US and Canadian DRIs. These were thyroid accumulation and turnover and iodine balance studies.

272B.—Use of a Non-radioactive Cadmium Affinity Assay for Metallothionein Content. Ashmead, S.D. Albion Advanced Nutrition, Clearfield, Utah, Hendricks DG, Utah State University, Logan, Utah.

Metallothionein has been identified as one of many probable regulatory proteins involved in the homeostatic control of zinc. This protein has been utilized to study zinc and a variety of methods have been developed for its purification and quantification. Radioactive cadmium (109Cd)/hemoglobin affinity assay was developed by Eaton and Toal(1) as a method for rapid and accurate determination of metallothionein content in biological tissues. We have modified the method of Eaton and Toal to assay for metallothionein content using a non-radioactive procedure. Male Sprague-Dawley rats were fed a controlled diet designed to deplete Zn stores in the intestinal epithelial cells followed by a period of repletion. At the end of the repletion period, sectionalized intestinal, liver, and kidney tissues were harvested and assayed for metallothionein content. Partially thawed tissue samples were cut and diluted four times by weight with cold 10 mM Tris buffer and then homogenized. The homogenate was centrifuged at 13,000 g for 10 minutes and then decanted. A 200 μL aliquot of the supernatant was incubated with a 2 ppm CdCl2 solution for 10 minutes. Then 100 μL of lyophilized bovine serum was added, mixed, boiled for 2 minutes, iced for 2 minutes, and centrifuged at 13,000 g for 2 minutes. This step was repeated. The resulting supernatant was analyzed for Cd content using ICP/MS based upon EPA method 6020 and calibrated using a 100 ppt Cd standard. It was found

that the samples had to be diluted further with nitric acid anywhere from 5–100 times depending on expected Cd content. Overall metallothionein content was detectable and quantified at levels that were appropriate to the tissue. This non-radioactive method shows appropriate sensitivity for biological tissues and warrants further investigation to correlate to standardized tests.

1. Eaton DL, Toal BF. (1982) Toxiclgy. Appl. Pharmacology 66:134–142.

273B.—Arsenic Exposure and Potential Health Risks from Domestic Well Water in Northern Alberta. Robert Audette,* Alex Mackenzie,[+] Weiping Chen[+] and Donald Schopflocher.[+] *Audette Consulting, St. Alberta, AB and [+]Health Surveillance Branch, Alberta Health and Wellness, Edmonton. AB, Canada.

In response to public concern about As levels in groundwater in Northeastern Alberta, a rigorous survey was undertaken in three Regional Health Authority (RHA) regions in Northern Alberta (Lakeland RHA—59 sites, Aspen RHA—51 sites and Keeweetinok RHA—38 sites) between January 1999 and June 2000. Within the individual RHAs, some of the chosen sampling locations were based upon previously collected historical information. Raw tap water from private domestic wells that are used for human consumption and food preparation was collected. A total of 2,817 of water samples were collected over a 13-month period. Arsenic along with 22 other trace metal elements was analyzed using a PE-SCIEXR Elan 6000 ICP–MS. Statistical analysis was performed on summary measures of As concentrations in relation to geographic areas and underlying bedrock geological formation variations, seasonal variation, and relationships to 22 other trace metal elements (Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Th, U, V, Zn). Summary Table of % of mean As (μg/L) in raw and treated water samples by regional health authority.

	Raw Water						Treated Water					
As Conc. (mg/L)	<5	5–9	10–24	25–29	30–49	>50	<5	5–9	10–24	25–29	30–49	>50
Lakeland RHA	39.1	10.9	28.1	6.3	15.6	–	37.1	20.3	26.8	5.8	9.8	0.2
Aspen RHA	84.3	7.8	3.9	3.9	–	–	75	6.8	17.6	0.6	–	–
Keeweetinok RHA	56.1	9.8	14.6	4.9	2.4	12.2	56.9	11.0	19.4	2.0	1.7	9.0

The principal findings for the characteristics of As in groundwater included: annual average As concentrations in excess of 25 μg/L (Canadian Drinking Water Guidelines, 2001) were observed in 21.9% of the raw water samples in Lakeland RHA, 3.9% in Aspen RHA, and 19.5% in Keeweetinok RHA; the As levels exceeded 50 μg/L in 12.2% of the Keeweetinok RHA raw water samples; no systematic seasonal variation of mean As concentration values was observed; elevated As concentrations in domestic well waters were associated with areas underlain by three bedrock geological formations (the LaBiche and Lea Park Formation and the Smoky Group) in Northern Alberta all of which are marine formations containing shale; elevated As concentrations were most likely to be observed in domestic well waters greater than 50 feet in depth; relatively strong relationships were observed among concentrations of As, Fe, and Mo; the sample sites from the areas underlain by the three bedrock geological formations appear to have different profiles of trace elements. The principal findings of As exposure and potential health risks included: the average intake of As from drinking water for the majority of residents in three RHAs falls within a typical Canadian intake; annual average exposure to levels of As from drinking local domestic well waters is considerably lower than the levels reported to cause adverse health effects in the scientific literature; although there is general consensus about the health risks posed by high concentrations of As in drinking water, there is no direct evidence on which to base risk estimation for levels of As at or below the current drinking water guidelines or standards.

274B.—Trace Element Removal During Chelation Therapy in Cardiac Patients. Robert Audette and Ellen Burgess. University of Calgary, Calgary, Alberta, Canada.

Many patients with coronary artery disease (CAD) report having Na-EDTA chelation, a popular alternative therapy, despite the lack of evidence of benefit. A double-blind trial was undertaken to assess the effectiveness of chelation therapy in patients with CAD. This companion study is the first to evaluate the removal of a number of trace elements with this chelator in a large number of patients. Eighty-four (84) patients were enrolled, 78 completed the 27-week therapy, and 58 underwent blood and urine testing for trace elements (30 received placebo treatments; 28 had Na-EDTA chelation). Patients had blood collected prior to the first, the 30th and 33rd (last) treatment; a 24-hour urine collection was made prior to the first treatment and on the first, 30th and 33rd days; a blood sample was collected at the end of each 24-hour urine collection. Using an Elan 6000 ICP-Mass Spectrometer, serum was analyzed for Al, Ba, Be, Cu, Mn, Ni, Sb, Se, V and Zn, blood for Cd, Co, Mo, Pb and Th, and urine for Al, As, Ba, Be, Bi, Cd, Cu, Hg, Mn, Pb, Sb, Se, Th, V and Zn. Some patients did not have levels within detectable limits for any or some of the trace elements studied; therefore the numbers of patients reported in an analysis may not equal the total number of patients in a group.

Summary Table for 24-hour Urinary Excretion of Trace Elements Pre and Post the First Intravenous Dose of Placebo or Na-EDTA:

	Placebo Pre	Placebo Post	Na-EDTA Pre	Na-EDTA Post	Difference in Effect
Pb (umol/D)	0.015 + 0.008 (n = 10)	0.096 + 0.169	0.017 + 0.012 (n = 12)	0.168 + 0.111	NS
Zn (umol/D)	7.2 + 3.2 (n = 29)	34.1 + 70.2	6.2 + 3.3 (n = 26)	153.1 + 90.9	$P < 0.0001$
Cd (nmol/D)	7.1 + 4.0 (n = 26)	12.1 + 3.0	7.3 + 3.5 (n = 26)	44.7 + 34.7	$P < 0.0001$
Mn (nmol/D)	33 + 27 (n = 20)	160 + 329	29 + 24 (n = 21)	433 + 230	P = 0.0036
Al (umol/D)	0.45 + 0.49 (n = 18)	0.43 + 0.41	0.20 + 0.11 (n = 16)	0.65 + 0.33	P = 0.0009

A significant difference in change from baseline existed only for Zn, Mn, Cd, and Al with the first dose. No significant difference was seen for Pb. There continued to be a significant increase in urinary excretion of Zn, Mn, and Cd at the 30th dose, but not at the 33rd dose. Chelation therapy with Na-EDTA for CAD was determined to be no different than placebo in clinical effect, but the trace element analyses demonstrates removal of Zn, thought to be an anti-oxidant, as well as Al, Cd and Mn.

277B.—Effect of Maturity on Cobalt Metabolism in Dairy Cattle. R.L. Kincaid, L. Lefebvre and J.D. Cronrath. Department of Animal Sciences, Washington State University, Pullman, WA 99164-6351.

Cobalt, an essential trace element in diets of ruminants, is utilized by micro-organisms in the rumen for the synthesis of vitamin B12 and numerous analogs of B12 (Young, 1979). Although some non-vitamin B12 roles for Co may exist, none have been elucidated. Diets of nonlactating, nonpregnant cows (n = 8) were supplemented with 17 mg of Co as cobalt glucoheptonate per day and effects on concentrations of Co in blood and liver determined. Cobalt supplementation did not affect Co in serum or liver, however, cows <3 yr of age had higher concentrations of liver Co than cows >3 yr (42 vs. 14 ng/g). The subcellular distribution of Co in liver was: cytosol, 38%; mitochondria, microsomal, and lysosome, 17% each; and nuclear, 12%. The concentration of Co in liver did not affect the relative subcellular distribution of Co. In a separate study, cows (n = 36) were fed 0, 12, or 25 mg supplemental Co from 21 d prepartum until parturition, then fed diets that contained 0.37, 0.68 or 1.26 ppm Co until 120 d post-partum. Cobalt supplementation did not affect concentrations of Co in serum, whole blood, or milk, however, the highest Co supplementation decreased ($P < .05$) liver Co. Compared to multiparous cows, primiparous cows had higher concentrations of Co in colostrum (93 vs. 119 ng/ml) and milk (94 vs. 99 ng/ml). Serum B12 concentrations were higher in primiparous than multiparous cows (1.81 vs 0.96 ng/ml), and serum B12 levels declined from 2.36 ng/ml in prepartum cows to 1.24 ng/ml in cows at 120 d postpartum. Similarly, serum Co decreased from 116 ng/ml at 7 d post-partum to 75 ng/ml at 120 d post-partum. These results indicate a gradual loss of Co and B12 with gestation and lactation in cows fed diets with typical Co concentrations, and that Co and B12 are not readily replenished by dietary Co supplementation. Reference: Young, R.S. 1979. Cobalt in Biology and Biochemistry. Academy Press, New York, p 197.

278B.—Effect of Supplementation with CENTRUM and Dietary Modification on Serum Lipoprotein Indices and Bioelements Concentrations in Cardiovascular Patients. Krejpcio, Zyman, Wójciak and Gaweçki. Department of Human Nutrition and Hygiene, August Cieszkowski Agricultural University, Poznan, Poland.

Cardiovascular disease is one of the main causes of increasing mortality in ageing populations. There are variety of complex factors playing role in etiology of heart disease. Zinc-copper hypothesis points out microelement imbalance as one of such factors. Since dietary copper intake is usually low in relation to zinc, their imbalance is likely to appear in modern population leading to increasing prevalence of cardiac disease. The objective of this study was to investigate the effect of moderate vitamin-mineral supplementation with CENTRUM pharmaceutical preparation and modification of diet on serum lipoprotein indices and bioelements concentrations in cardiovascular patients. 12 patients, over 50 years old (6 men and 6 women) suffering from cardiovascular disease, were subdivided into two groups (3 men and 3 women, each) and were subscribed for dietary modification. The first group (C) was given a complex vitamin-mineral supplement CENTRUM (American Cyanamid Company, Pearl River, USA), one tablet per day, while the second group (D) was advised to include into daily diet food products rich in dietary zinc and copper (like: fish, liver, seeds, nuts, legumes) for 30 consecutive days. The serum LDL, HDL, total-cholesterol and TG concentrations were determined with the use of standard methods by Clinical Laboratory, while the serum Ca, Mg, Zn and Cu concentrations were determined by AAS method. All measurements were done in the first and last day of the study. It was found that: at the beginning of the study cardiovascular patients had significantly increased serum total-cholesterol, LDL, TG and serum Zn, and Zn/Cu ratio, while serum Mg was lower in comparison with the reference values. Dietary supplementation with CENTRUM and modification of diet significantly influenced some serum lipoprotein indices and serum Ca, Mg, and Zn in patients with cardiovascular disease. Serum total cholesterol, HDL and LDL decreased by 24%, while serum Ca and Mg increased by 20–23%. On the other hand, serum Zn and Zn/Cu ratio decreased drastically by 70% after 30 days of dietary intervention. The degree of serum indices alterations was comparable in both groups, and did not depend on the type of dietary intervention nor gender of patients with cardiovascular disease. Increased intake of natural food products rich in Zn, Cu and other minerals as well as variety of accompanied nutrients seems to be beneficial for regulation of lipid and mineral balance in cardiovascular disease and as effective as supplementation with pharmaceutical preparation.

279B.—Serum Trace Element Levels (Cu, Zn, Se) in a Sample of the Human Population of Lisbon. P.A. Lopes,* M.C. Santos,[+] L. Vicente,* O. Rodrigues, M.L. Pavão,[++] J. Nève[ϕ] and A.M. Viegas-Crespo.*** *C.B.A./Dept. Zoology, Fac. Sciences, Univ. Lisbon, 1749-016 Lisbon, Portugal; [+]C.E.B.F./Dep. Chem. and Biochem, Fac. Sciences, Univ.

Lisbon, 1749-016 Lisbon, Portugal; **National Health Inst. Dr. Ricardo Jorge, 1649-016 Lisbon, Portugal; ++Dept. Technol. Sci. and Develop., Univ. Azores, 9500 P. Delgada, Portugal; ϕInst. Pharm, Free Univ. Brussels, C. Plaine, B-1050 Brussels, Belgium.

Some trace elements are accepted as essential for optimum human health because of their diverse metabolic characteristics and functions. Copper, zinc and selenium are essential trace elements with important functions related to the antioxidant biological systems. The main purpose of this study was to evaluate the serum selenium, copper and zinc levels in Portuguese subjects living in the city of Lisbon. Two hundred twenty eight volunteer healthy subjects of both sexes, aged 20 to 70 years were analyzed according to sex and/or range age. The relationship between these trace element levels and tobacco consumption was also considered. Copper and zinc were analyzed by flame atomic absorption spectrometry and selenium by electrothermal atomic absorption spectrometry. The results of serum trace element levels (M ± SD) are summarized in the following table:

	Women	Women	Men	Men
Age range	20–44 (n = 97)	45–70 (n = 70)	20–44 (n = 41)	45–70 (n = 20)
Copper (μg/dL)	139 ± 56.8	123 ± 32.7	99.5 ± 41.7	101 ± 26.9
Zinc (μg/dL)	97.7 ± 16.7	100 ± 15.6	106 ± 17.1	103 ± 16.9
Selenium (μg/L)	75.4 ± 13.3	84.5 ± 14.9	82.3 ± 18.8	88.8 ± 14.7

Trace element levels did not change with age. Serum concentrations of these elements were in the same range than those found for populations of other European countries. Differences between sexes were observed, since serum zinc and selenium levels were slightly higher in males than in females for the younger group. The copper levels were higher in females as compared to males for all age range. These data can be explained by the hormonal status and/or oral contraceptive steroids intake, particularly for copper. The results concerning tobacco consumption will be further discussed. This study was supported by the project PRAXIS/PSAU/C/SAU/66/96.

280B.—Trace Element Levels in Freshwater Fish (Leuciscus alburnoides Complex) and in Field Mice (Mus spretus) from a Mine Area. P.A. Lopes,* M.T. Pinheiro, M.L. Mathias,* M.J. Collares-Pereira,* M.C. Santos+ and A.M. Viegas-Crespo.*** *Centro de Biologia Ambiental and Departamento de Zoologia, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, +Centro de Estudos de Bioquímica e Fisiologia and Departamento de Química e Bioquímica Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal, **Departamento de Física, Instituto Tecnológico e Nuclear, Sacavém, Portugal.

Many studies refer to the possible hazard caused by the accumulation of heavy metals in living organisms. Some metals and other elements are required in small but critical amounts by most animals for their growth, although increased concentrations can caused toxicity. This study reports on the response of wild species of freshwater fish and mice to the environmental pollution derived from Cu mineral extraction at the South East of Portugal. Both fish and mice populations were referred to reference populations inhabiting at a natural park classified as a reference area. The Mn, Fe, Cu, Zn and Se concentrations were determined in the liver of fish and mice populations collected along one year at the polluted and at the reference areas using PIXE (Particle Induced X-ray Emission) analytical technique. The environmental quality was also assessed. The major alterations observed were for the enhanced Cu and Se liver concentrations in fish and for the increased Fe and Se liver concentrations in mice, from the polluted area. At the mine area minimum and maximum concentrations of Cu in fish liver were of 26 mg/kg wet tissue and 34 mg/kg wet tissue, respectively, whereas Cu concentrations in the reference site ranged between 5.7 to 7.8 mg/kg wet tissue, along the year. Identical increase of liver concentration was observed for Se in exposed fish populations by report to the reference population: 2.4 to 5.7 mg/kg wet tissue versus 0.5 to 1.0 mg/kg wet tissue, respectively.

For the mice populations smaller variations were observed in trace element liver concentrations than those found in fish. The Fe liver content was enhanced at the polluted area when compared with data from the reference area: 310 to 930 mg/kg wet tissue and 320 to 660 mg/kg wet tissue, respectively. Se liver contents were also increased in mice from the mine area in relation to those from the unpolluted site: 1.6–3.4 mg/kg wet tissue and 0.9 to 1.6 mg/kg wet tissue, respectively.

The distinct responses of both animal models may result from different elemental availability in the environment and also from their different physiology.

286B.—Assessment of the Selected Bioelements Levels in Women Organism Based on Hair Analysis. Wójciak, Krejpcio and Olejnik. Department of Human Nutrition and Hygiene, August Cieszkowski Agricultural University, Poznań, Poland.

During different carcinomas the levels of some trace elements in cancer tissues as well as in whole body may change. In the organism of women, especially living in industrial countries, breast cancer is the most common type of cancer. Early detection and mastectomy usually leads to complete recovery. The question is: How long the human organism return to the complete balance? In this study we tried to find possible differences between selected hair metals concentrations in two

groups of women aged 37–72 (mean age 57), 35 subjects after mastectomy (MG) and 35 control subjects (CG). Each woman was healthy in opinion of their physicians. We measured hair Ca, Mg, Zn, Cu and Fe levels by flame atomic absorption spectrometry method. The MG had significantly lower hair Ca, Mg and Zn concentrations than HG (1334, 67, 225 vs. 1871, 109, 297 g/g, respectively), and higher hair Fe concentration (52 vs. 33 g/g, respectively). The hair Cu levels didn't differ between both groups. We also evaluated the influence of time after mastectomy on hair metals levels in the studied subjects. The MG was divided into three subgroups based on the time after mastectomy procedure: A. below 2 years (66%), B. 2–5 years (23%), C. above 5 years (11%). The hair Ca, Mg, Zn and Cu levels in MGC were significantly lower than in MGA and MGB, however there were no differences between MGA and MGB. Although, the hair Fe level was higher in MGC than in MGA and MGB, we didn't find significant differences between MGA and MGB. We conclude that breast cancer may produce some mineral deficiencies in the long run. Therefore mineral status of women after mastectomy procedure should be monitored and supplementation should be included into common practice, if necessary.