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Abstracts*)

Advances and Controversies in B-Vitamins and Choline

Leipzig, Germany, March 5-8, 2012

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Abstracts

INVITED SPEAKER

INV 01
H_2S Homeostasis and the Transsulfuration Pathway

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Despite the excitement about the discovery of a third gaseous signaling molecule and the consequent profusion of literature on H_2S biology, progress in the field has been impeded by the lack of sulfide-specific sensors for detection/imaging, and an understanding of how cells maintain very low steady-state levels of H_2S, amplifying the signal as needed. The enzymes of the transsulfuration pathway, cystathionine b-synthase (CBS) and cystathionine g-lyase (CSE) are important for the endogenous production of H_2S. We have quantified CBS and CSE levels in murine liver, brain and kidney and shown that at physiologically relevant concentrations of substrate, and adjusting for the differences in CSE versus CBS, CSE is the primary source of hepatic H_2S. We will discuss new data on estimation of hepatic sulfur flux both through H_2S biogenesis and O_2-dependent H_2S catabolism, which are very high and comparable to the flux of sulfur into the antioxidant pool, glutathione. These data implicate regulation of H_2S clearance as a potentially important "off-switch" in H_2S signaling. We will also discuss advances in H_2S detection and imaging via a collaborative study with the He laboratory (University of Chicago), which has developed sulfide-specific fluorescent sensors.

INV 02
Cystathionine gamma-lyase dependent H_2S formation in perivascular adipose tissue in experimental metabolic syndrome

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Background: Perivascular adipose tissue (PAT)-derived H_2S decreases vascular tone and inhibits atherogenesis. Obesity is associated with impaired anticontractile effect of PAT, however, effect of obesity on H_2S has not been studied.
Aim: To examine the effect of experimental metabolic syndrome on cystathionine γ-lyase (CSE)-H_2S system in PAT.
Methods: Obesity was induced by feeding rats a calorie diet for either 1 (O1) or 3 (O3) months. H_2S production by isolated PAT was measured by electrochemical sensor, and phenylephrine-induce contractility of aortic rings with or without PAT was examined.
Results: Both O1 and O3 groups were characterized by increased adiposity and plasma leptin, but only O3 group was hyperinsulinemic and insulin-resistant. H_2S production by PAT and anticontractile effect of PAT on aortic rings were augmented and impaired in the O1 and O3 groups, respectively. CSE expression and activity in PAT was normal in the O1 but reduced in the O3 group. Inhibitor of mitochondrial H_2S metabolism, stigmatellin, increased H_2S production by PAT to a lesser extent in the O1 than in the control group, suggesting impaired H_2S oxidation in obese rats. This effect most likely resulted from adipose tissue hypoxia observed in obesity. Insulin sensitizer, rosiglitazone, increased CSE expression and activity as well as H_2S production in the O3 group but had no effect in either lean or O1 animals.
Discussion and Conclusions: Early obesity, not associated with hyperinsulinemia/insulin resistance, results in overproduction of H_2S in PAT because mitochondrial oxidation of the gas is reduced. In contrast, in more advanced metabolic syndrome when insulin resistance appears, H_2S production is impaired due to reduced CSE expression.

INV 03
Novel defects in folate and homocysteine metabolism – new views on homocysteine metabolism

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Over the last year we discovered two novel inborn errors of folate and homocysteine metabolism: dihydrofolate reductase (DHFR) deficiency and adenosine kinase (ADK) deficiency.
The clinical and biochemical presentations of both defects will be described and their impact on folate and homocysteine metabolism discussed.
Both defects provide new insights in regulation of this metabolism, and their importance in health and disease.

INV 04
Choline and the brain

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Choline is an essential nutrient for humans. Metabolically choline is used for the synthesis of phospholipids, and as a precursor of the neurotransmitter acetylcholine. Following oxidation to betaine, choline functions as a methyl group donor, and thus its supply influences DNA and histone methylation – two central epigenomic processes that regulate gene expression. Studies in rodents have shown that high choline intake during gestation and the perinatal period improves cognitive function in adulthood, prevents age-related memory decline, and protects the brain from damage and functional deterioration associated with epilepsy and other insults (e.g. fetal alcohol syndrome). The behavioral changes are accompanied by modified patterns of expression of cortical and hippocampal genes including those encoding key proteins that contribute to the mechanisms of learning and memory. These actions of choline correlate with cerebral cortical changes in global- and gene-specific DNA cytosine methylation and with changes of the methylation pattern of lysine residues 4, 9 and 27 of histone H3. Moreover, gestational choline modulates the expression of DNA- and histone methyltransferases. In addition to the central role of DNA and histone methylation in brain development, these processes are highly dynamic in adult brain, modulate the expression of genes critical for synaptic plasticity, and are involved in mechanisms of learning and memory. A recent study documented that in a cohort of normal elderly people, verbal and visual memory function correlated positively with the amount of dietary choline intake, further supporting the idea that adequate choline nutrition is essential for the maintenance of cognitive function in aging humans.

INV 05
Maternal choline intake in humans alters metabolic, epigenomic and epigenetic marks

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The effect of varied maternal choline intake on metabolic, epigenomic and epigenetic readouts in humans is unknown. We recently conducted a 12-wk controlled feeding study in which pregnant women were randomized to choline intakes of either 480 or 930 mg/d throughout their third trimester. The higher maternal choline intake yielded greater use of choline as a methyl donor in both maternal and fetal compartments (Yan et al. 2011, Submitted) and influenced the epigenomic state of the placental tissue. In addition, varied maternal choline intake altered the epigenetic state of genes that regulate fetal HPA axis activity and consumption of 930 versus 480 mg/d yielded lower concentrations of the HPA axis product, cortisol, in venous cord blood (Jiang et al. In Preparation). These data suggest for the first time in humans that maternal intake of the methyl donor, choline, may modulate fetal programming with possible long-term effects on health.

INV 06
Choline intake and cancer risk: epidemiologic studies

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Background: Choline and betaine are involved in methyl group metabolism as methyl donors and, thus, may be related to a reduced cancer risk. Choline is also a precursor for cell membrane phospholipids, phosphatidylcholine and sphingomyelin.
Aim: Few epidemiologic studies have examined intakes of these nutrients in relation to risk of cancer. We have investigated choline intake in relation to risk of colorectal adenoma, which is a precursor for colorectal cancer, colorectal cancer, and breast cancer in large prospective follow-up studies.
Methods: We examined intake of choline and betaine and risk of colorectal adenoma, colorectal cancer, and breast cancer among women in the Nurses' Health Study and men in the Health Professionals Follow-up Study. Nutrient intake was assessed with a validated food-frequency questionnaire multiple times since baseline.
Results: Contrary to expectation, choline intake was associated with an elevated risk of colorectal adenoma. Betaine intake had a non-linear inverse association with risk of colorectal adenoma. Among individual sources of choline, choline from phosphate-dylcholine and from sphingomyelin were each positively related to adenoma risk. However, we did not find any association between choline and betaine intake and colorectal cancer risk. We also found that higher choline intake was not associated with premenopausal breast cancer risk but was associated with a modest elevated risk of postmenopausal breast cancer.
Discussion: We found no evidence that higher intakes of choline and betaine reduce risk of colorectal adenoma, colorectal cancer and breast cancer. Findings from these studies and other studies on intakes of choline and betaine and cancer risk in humans will be discussed.

INV 07
Role of Vitamin B6 and its Metabolite in Cardiac Dysfunction in Ischemic Heart Disease

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Although plasma level of vitamin B in patients with myocardial infarction (MI) was depressed, the role of vitamin B in ischemic heart disease is poorly understood. Since cardiac dysfunction in MI is associated with a defect in purinergic system, the effects of vitamin B and its metabolite, pyridoxal-5'-phosphate (PLP) were examined by employing an experimental model of MI in rats. Unlike vitamin B, treatment of MI animals with PLP for 4 to 6 weeks was found to attenuate the MI-induced cardiac dysfunction. Treatment of isolated hearts with PLP, unlike vitamin B, also attenuated the ischemia-reperfusion induced myocardial dysfunction. These beneficial effects of PLP appear to be due to depression in Ca2+-influx and subsequent attenuation of intracellular Ca2+-overload because ATP-induced increase in [Ca2+]i in cardiomyocytes and specific ATP-binding to the sarcolemmal membrane were decreased by PLP. Although vitamin B deficiency in rats for a prolonged period was also observed to decrease the ATP-induced increase in [Ca2+]i in cardiomyocytes, the KCl-induced increase in [Ca2+]i was markedly augmented. These results indicate that vitamin B may exert its cardioprotective effects by reducing the intracellular Ca2+ through the formation of its metabolites. (The infrastructure support for this project was provided by the St. Boniface Hospital Research Foundation.)

INV 08
Folic acid and homocysteine: from coronary vasodilation to neuroprotection

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Background: Numerous studies have linked folate deficiency with risk of arterial disease, neurodegenerative diseases, dementia and seizures. It is of interest whether dietary supplements of folic acid can improve vascular or neural function of subjects at risk.
Aims and Methods: The first aim of study was to assess the effects of folic acid administration (100 μM) on coronary flow and oxidative stress markers (nitrite outflow, superoxide anion, TBARS) with or without L-NAME (NO synthase inhibition, 30 μM) in isolated rat heart. The second aim was to examine the effects of folic acid administration (5 mg/kg, 10 mg/kg, 15 mg/kg, i.p, respectively), on behavioral and electroencephalographic characteristics of DL-homocysteine thiolactone-induced seizures (8 mmol/kg, i.p) in adult rats.
Results: The findings of the first study are: 1. folic acid induced rat coronary vasodilation, 2. it was accompanied by significant increase in nitrite outflow, decreased superoxide anion production, and (surprisingly) increased TBARS production, 3. L-NAME did not change significantly folic acid-induced responses. The findings of second study are: 1. there were no behavioral and EEG signs of seizure activity in groups of rats treated by folic acid alone, 2. however, following high dose of folic acid administration (15 mg/kg) the incidence of seizures were significantly lower and latency was significantly prolonged.
Conclusions: The obtained findings suggest that folic acid possess vasodilatory, antioxidative and anticonvulsive properties in adult rats.

INV 09
Inflammatory markers, kynurenines, and B-vitamins in relation to chronic disease and mortality in a prospective population based study

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Background: It is hypothesized that inflammation plays a key role in the onset of many chronic diseases. Although inverse associations of vitamins B6 and B2 with CVD and cancers are shown, mechanisms are unclear. However, studies have revealed lower vitamin B6 levels with increased C-reactive protein (CRP) - a commonly used inflammatory marker as well as neopterin and the kynurenine/tryptophan ratio (KTR). Inflammation increases levels of neopterin and also the conversion of tryptophan to kynurenine, which is further metabolised using vitamins B6 and B2 as cofactors. The combination of low plasma vitamin B6 and presence of inflammation has recently been linked to high 3-hydroxykynurenine, a metabolite downstream of kynurenine. The vitamins B6 and B2 may form a link between the kynurenine pathway and CVD and cancer
Aim: To prospectively investigate associations of vitamins B6 and B2, kynurenine metabolites and inflammatory markers with cancer and CVD incidence, and mortality.
Methods: Blood samples within The Hordaland Health study (HUSK, n=7.047) were taken in 1997-1999 in which we measured vitamin B2 (riboflavin and flavin mononucleotide) and B6 (pyridoxal' 5-phosphate, pyridoxal and 4-pyridoxic acid) species, kynurenines (kynurenine, kynurenic acid, anthranilic acid, 3-hydroxy-kynurenine, xanthurenic acid, 3-hydroxyanthranilic acid), and inflammatory markers (CRP, neopterin, KTR). The HUSK study has been linked to the Cardiovascular Disease Registry Health Region West (data through 2008; n=2185), the Cancer Registry of Norway (data through 2007; n=746), and the Cause of Death Registry (data through 2008; n=851).
Results: Data are currently being analysed, and results will shed light on major gaps in the research field of inflammation, kynurenines, and B-vitamins in chronic inflammatory diseases.
Discount and conclusions: This is the first prospective study focusing on the combination of inflammatory markers, several kynurenines and B-vitamins in relation to inflammatory diseases.

INV 10
Immune activation and moderate hyperhomocysteinemia

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Moderate hyperhomocysteinemia is often associated with inflammation and immune activation. In vitro, stimulated peripheral blood mononuclear cells release homocysteine. This effect is suppressed by anti-inflammatory drugs like aspirin, salicylic acid and atorvastatin. Likewise, in various diseases including coronary artery disease (CAD), rheumatoid arthritis, certain types of cancer and various forms of dementia but also in older aged healthy normals, significant correlations between increased concentrations of homocysteine and higher concentrations of immune activation markers like neopterin or soluble TNF-receptors have been documented [1]. B-vitamin supplementation in patients was found to decrease homocysteine concentrations but did not influence immune activation markers, as it had no drastic influence on the clinical performance.
In patients after multiple trauma and with sepsis, total homocysteine concentrations increase during follow-up. This is preferentially true in non-survivors but not in survivors [2]. Because the patients receive standardized parenteral nutrition after the end of hypodynamic shock, different supply with vitamins is unlikely to be the reason for the increase of homocysteine, rather it is associated with a stronger pro-inflammatory response. This study further supports the conclusion that underlying immunopathogenetic mechanisms may contribute to the development of moderate hyperhomocysteinemia in various diseases. Because of an increased demand of B-vitamins in inflammatory conditions, B-vitamins like folate, B12 and B6 may need to be supplemented.

[1] Schroecksnadel K, et al. Curr Pharm Biotechnol 2004;5:107-18.
[2] Ploder M, et al. Mol Med 2010;16:498-504.

INV 11
The role of epigenetics in neurodegeneration: the Alzheimer's Disease paradigm

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Neurodegeneration is the progressive loss of neurons function, generally resulting in cell death and neurodegenerative diseases. The greatest risk factor for neurodegenerative diseases is aging, which is influenced by physiological and environmental stimuli. Epigenetic modifications can mediate environmental insults that a person encounters during his life. As a matter of fact, epigenetic mechanisms can be considered as a link between environment and its effect on the genome. Advances in epigenetic research stressed the involvement of epigenetics in different neurodegenerative diseases.
In the last years, my laboratory was aimed at studying the role of DNA methylation in Alzheimer's Disease (AD), the most representative among neurodegenerative diseases.
To this scope, we analyzed methylation of genes associated to the disease in a murine AD model and, very recently, in AD patients.
We found that PSEN1 gene, involved in amyloidogenesis, is regulated by methylation in hyperhomocysteinemic mice (a condition exacerbating AD-like features) and in brains of AD patients.
Our results demonstrate the existence of specific mechanisms by which epigenetics affects the progress of neurodegeneration. Moreover, we evidenced that DNA methylation could be underestimated in the current studies.
Besides the new insights in the comprehension of complex diseases, the most appealing part of this story is that epigenetic modifications are reversible, suggesting that epigenetic drugs to be used in disease treatment.

INV 12
New insights into cobalamin absorption and metabolism using accelerator mass spectrometry

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Much of our understanding of cobalamin (Cbl) derives from radiocobalt tracer studies. We now study Cbl absorption and metabolism with unprecedented resolution using a novel technique developed by our group in which 14C is incorporated into the lower axial ligand of cyanoCbl (14C-cyanoCbl) through bacterial synthesis (Proc Nat Acad Sci 2006;103:5694-9). Following oral 14C-cyanoCbl, blood was analyzed for 14C by accelerator mass spectrometry (AMS). The plasma response was consistent with the expected behavior of oral Cbl. 14C reached a peak level within 6-8h, and was bound to the physiological transport protein, transcobalamin (TC). We also reported surprising degradation of 10-50% of the tracer dose which was excreted in the urine, 100-fold greater than in previous reports using 57Co-labeled cyanoCbl (0.1-0.5%). We have analyzed the plasma clearance of TC-bound 14C-Cbl. Following oral 14C-cyanoCbl (1.3 µg, 50 nCi), blood specimens were obtained and the TC-bound fraction of plasma 14C appearing in plasma at timed intervals was determined by an immunoaffinity method. The mean apparent plasma TC-bound 14C-Cbl half-life in 9 normal subjects was 4.3h, longer than previously published values that ranged from 6 min to 1.5h. We also studied a patient with pernicious anemia. A small peak of 14C was identified in the plasma 5h following the oral dose, earlier than observed in the control subjects. However, when the immunoaffinity purified TC-bound fraction was examined for 14C, none was detected, suggesting that the non-TC 14C was a degradation product of the 14C-cyanoCbl. For improved clinical reliability, plasma measurement of tagged Cbl in an absorption test should therefore be accompanied by purification of the TC-bound fraction.

INV 13
Epigenomics effects of deficiency in methyl donors

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Background: The deficiency in methyl donors, folate, vitamin B12 and choline influences the risk to develop complex degenerative diseases
Aim: To dissect the consequences deficiency in methyl donors related with epigenomic mechanisms in brain, myocardium and liver, at different steps of life
Methods: We studied cellular models (NIE-115 cells with stable expression of transcoblamin fused to oleosine, with sequestration of vitamin B12 and decreased methionine synthase and folate deficient H19-7 cells) and rat models with deficiency during pregnancy and aging
Results: Deficiency in methyl donors suppresses foetal growth in relation with Insulin-like growth factor 2, H19 and Ghrelin impaired expression. Expression of chimeric protein produces slower proliferation and speedier differentiation of neuroblastoma cells through altered expression of PP2A, proNGF, and TACE and apoptosis and Parkinson-like phenotype by transfecting rat *substantia nigra*. Altered differentiation of neuronal progenitors is observed in H19-7 cells and in specific brain areas of deficient pups, with maintained tissue and behaviour anomalies during aging. Expression of proteins involved in neuroplasticity is decreased in hippocampus and cerebellum of females, in relation with epigenomic dysregulation of ERa pathways. It leads to cardiac hypertrophy and liver steatosis in relation with decreased expression of PPARa and ERRa and inactivation of PGC1a by imbalanced hypomethylation/acetylation and subsequent impaired mitochondrial fatty acid oxidation
Discussion – conclusion: The deficiency in methyl donors influences the risk to develop complex degenerative diseases by epigenetic and epigenomic mechanisms related with the foetal programming hypothesis

INV 14
B-Vitamin Dependent Methionine Metabolism in Pathogenesis, Prevention, and Treatment of Alcoholic Liver Disease

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Question: Do deficiencies of folate and B6 regulate the pathogenesis of ALD through their effects on hepatic methionine metabolism?
Methods and results: Ethanol fed micropigs fed a folate deficient diet developed ALD in association with reduced levels of liver SAM and increased SAH with increased expressions of selected liver injury genes that were prevented by SAM. Intragastric ethanol fed mice developed ALD with reduced SAM:SAH ratios and increased expressions of genes associated with altered histone lysine patterns. A clinical study of chronic alcoholics with and without ALD found decreased serum folate levels and low B6 levels in ALD patients that correlated with increased serum cystathionine, consistent with reduced activity of B6-dependent cystathionase. A controlled 6-month clinical trial of supplemental SAM had no effect on liver histology in ALD while serum B6 levels were unchanged.
Conclusions: Aberrant B6 and folate dependent hepatic methionine metabolism is found in clinical and experimental ALD, is consistent with altered epigenetic gene regulation, and may be prevented but not treated by SAM supplementation.
References: [1] Halsted CH et al, Proc Nat Acad Sci 2002; 99:10072-7; [2] Esfandiar F et al, Am J Physiol 2005;289:G54-63; [3] Villanueva J et al,Alcohol Clin Exp Res 2007;31:1934-43; [4] Esfandiari F et al, Hepatology 2010; 51:932-41; [5] Medici V et al, J Hepatology 2010; 53: 551-57; [6] Medici V et al, Alc Clin Exp Res 2011, In Press.

INV 15
Insights into Functional Cobalamin Deficiency: The *cblC* Proteome

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Background: Mutations in the MMACHC gene disrupt processing of the upper-axial ligand of newly internalized cobalamins, leading to a functional deficiency of the vitamin and the cblC disorder. The full spectrum of protein changes that accompany human functional cobalamin deficiency is unknown.
Aim: We hypothesized that the proteome of skin fibroblasts from cblC patients will differ from that of normal subjects, and that these differences may correlate with the clinical manifestations of the disease.

Methods: To test this hypothesis, the proteomes of normal and cblC fibroblasts were quantitatively examined by two dimensional difference in-gel electrophoresis and liquid chromatography-electrospray ionization-mass spectrometry. The effect of hydroxocobalamin supplementation on the cblC proteome was also assessed.
Results: The cblC fibroblasts exported increased levels of both homocysteine and methylmalonic acid compared to normal fibroblasts. The cblC cells also had decreased levels of total intracellular folates. Major changes (>2-fold) were observed in the expression levels of proteins involved in cytoskeleton organization and assembly, the neurological system, and cell signaling. Supplementation with hydroxocobalamin did not restore the cblC proteome to the patterns of expression observed in control cells.
Discussion and Conclusion: The metabolic profile of culture dcblC fibroblasts is consistent with the clinical phenotype of functional cobalamin deficiency in vivo. Pathway analysis of the differentially expressed proteins demonstrated strong associations with those implicated in neurological, muscular, skeletal and cardiovascular disorders in the cblC cell lines. Altogether, these results concur with the observed phenotype of patients with the cblC disorder and their variable response to treatment with hydroxocobalamin.

INV 16
Osteoporosis – New Diagnostic and Clinical Aspects

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Osteoporosis is one of the leading age related diseases with 2.2 million Australians being affected. Fragility fractures represent the most frequent complication in these patients and are associated with substantial morbidity and mortality. However, osteoporosis is still a largely under-recognized disease and accounts for less than 1% of all problems managed by general practitioners.
In recent years substantial progress has been in made in understanding the pathomechanisms leading to bone loss and osteoporosis. Besides classical age related factors, such as immobility, sex hormone deficiency and vitamin D deficiency additional factors including bone marrow fat and hyperhomocysteineaemia have been established. In addition, there is substantial data supporting a mechanistic link between bone and energy metabolism, which sheds further light on the inverse relationship between fracture risk and body weight.
Although the diagnosis of osteoporosis is still based on a detailed history, the measurement bone mineral density and long known biochemical tests, significant advances have been made in utilizing this information more rationally. For example, osteocalcin is traditionally known as a bone formation marker. Today we know that osteoclastic bone resorption significantly contributes to circulating osteocalcin levels. Furthermore, there is mounting evidence that OC is a hormone like molecule with important metabolic functions.
Similar to cardiovascular disease treatment decisions are increasingly based on individual fracture risk using risk calculators, such as the FRAX or the Garvan Institute calculator. Advances have also been made in the therapeutic field. Besides classical agents, such as bisphosphonates a number of new drugs have been introduced or are currently being explored including the RANKL inhibitor denosumab or the cathepsin K inhibitor odanacatib.
The aim of this talk is to provide an overview about new mechanistic, diagnostic and clinical aspects of osteoporosis.

INV 17
Plasma phospholipids in diabetic and elderly subjects are related to C1-metabolism: possible impact on disease risk

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Background: Type 2 diabetes mellitus is associated with higher risks for coronary heart disease, polyneuropathy, nephropathy, and neurodegenerative diseases. The underlying mechanisms are not well understood. Low B-vitamins and hyperhomocysteinemia are common in diabetic patients. Changes in methyl group metabolism and medications might affect phospholipids (PL) in diabetics and this might be related to clinical outcomes. Unsaturated fatty acids, decreases in lysophosphatidylcholine (LPC) and sphingomyelin (SM) concentrations have been linked to diabetes and alterations in SMs and ceramids might play a role in amyloidogenesis and inflammatory stress related neuronal apoptosis. Our study is concerned with alteration in PL metabolism in diabetics and elderly subjects.
Methods: The study included 92 type 2 diabetics and 67 controls. Additionally, a double blind intervention study over one year on 60 elderly people with either 500 µg folic acid, 500 µg vitamin B12, 50 mg vitamin B6, 1200 IU vitamin D and 456 mg Ca++ /day; or with 1200 IU Vitamin D and 456 mg Calcium/day. We measured B-vitamin markers (fasting Hcy, MMA, folate, B12, holoTC), and blood methylation markers [S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH), choline, betaine]. Concentrations of numerous species of phospholipids [phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), and sphingomylein (SM)] were measured in plasma.
Results: Compared to controls lower concentrations of certain species of LPC and SM were found in diabetics. Diabetics treated with statins displayed compared to those without statins significantly lower concentrations of certain species of LPC and SM. Diabetics on metformin had significantly decreased concentrations of total and certain species of LPC and PE compared to those without metformin. We also found significantly higher ratio of methylated PL to non-methylated PL in metformin treated diabetics. Certain species of SM and LPC showed significant correlations to markers of inflammation, renal function and glucose metabolism. Backward regression analysis revealed that betaine and choline were independent variables explaining significant changes in phospholipids concentrations. The one-year intervention trial with B and D vitamins resulted in significant alterations of some phospholipid species.
Conclusion: It is concluded that changes in phospholipids might be triggered by alterations of methyl donors (folate, choline, betaine) and might be important for the development of age related diseases like diabetes, inflammation, neurodegeneration or renal dysfuntion. Further studies should provide information about the usefulness of certain phospholipid species as biomarkers for the above discussed diseases.

INV 18
Metabolic and Functional Effects of Choline in Children with Cystic Fibrosis

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Although considerable advances in care of patients with Cystic Fibrosis (CF) have led to an increased lifespan and life quality, CF remains a complex disease with many clinical complications,

including liver disease, that are incompletely understood. This is emphasized by a wide variability in complications and their severity among CF individuals with the same mutation. Other factors must, therefore, converge to exacerbate or minimize the underlying CF gene defect. Nutrition is key among these variables. Choline and methionine are major dietary methyl sources, but methionine also provides sulphur, crucial for protein, cysteine, glutathione and taurine, while choline functions in phospholipids and acetylcholine. Plasma choline, and its metabolites betaine and dimethylglycine are reduced, but malabsorption of choline containing lipids and bile acids is increased in CF children. We conducted a prospective 6 month intervention with 2 g/day choline bitartartate in 29 CF children. Cross-sectional studies with 45 children without CF and 84 children with CF were done to separate effects of age and disease on choline and its metabolites. Lower plasma choline, betaine and dimethylglycine in children with CF were increased, and homocysteine was decreased by choline supplementation. A subset of children with CF responded to choline with a lowering of blood pressure and improved pulmonary function, measured by spirometry. Urinary glycine excretion was reduced, but polyamine excretion was increased in CF children. Choline improves but does correct the methyl and sulphur amino acid abnormalities in CF, possibly due to continuing malabsorption of glycine and methionine which interact with choline metabolism.
Funded by Cystic Fibrosis Fdn.

INV 19
Homocysteine in alcoholic liver disease

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Background: Although hyperhomocysteinemia is associated with alcoholic liver disease, the molecular mechanisms for the production of elevated homocysteine (Hcy) are largely unknown.
Aim: We hypothesized that reactive oxygen species, produced as a result of EtOH-induced upregulation of cytochrome P450 2E1 (CYP2E1), results in the inactivation of B12-dependent methionine synthase (MS) and/or the cblC protein MMACHC, a B12 processing enzyme.
Methods: To test the hypothesis in vitro, we utilized HepG2 cells transfected with CYP2E1 (E47) and control cells transfected with empty vector (C34) both kindly provided by Dr. Arthur Cederbaum. Intracellular B12 was determined by HPLC after feeding [^{57}Co]-cyanocobalamin. To test the hypothesis in vivo, we determined hepatic MS enzyme activity in wild type and CYP2E1 null mice (CYP2E1$^{-/-}$) fed a diet with increasing levels of EtOH for 25 days.
Results: MS activity was 18% lower in E47 compared to C34 cells prior to EtOH treatment, and was further reduced by 15% in E47 after EtOH treatment. EtOH had no effect on MS activity in non-CYP2E1-expressing C34 cells. There was a concomitant increase in Hcy in E47 cells with low MS activity, and a dramatic decrease in glutathione in untreated and EtOH-treated E47 cells. Intracellular B12 profiles were similar in both C34 and E47 with or without EtOH. In wild-type mice, there was a 60% decrease in hepatic MS activity after EtOH feeding. In contrast EtOH had no effect on hepatic MS activity in CYP2E1$^{-/-}$ mice.
Discussion and Conclusion: CYP2E1 expression, in the absence of EtOH, was associated with reduced MS activity, increased Hcy and reduced glutathione in E47 compared to C34. Thus, MS enzyme activity is significantly reduced in CYP2E1-expressing cells bothin vitroandin vivo. The B12 processing enzyme MMACHC appears to be unaffected by CYP2E1 expression and EtOH treatment.

INV 20
Cellular damage by homocysteine: the role of protein N-homocysteinylation

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Protein-related homocysteine (Hcy) metabolism generates: 1) a chemically reactive thioester, Hcy-thiolactone; 2) Hcy-modified protein, *N*-Hcy-protein; and 3) an isopeptide, *N*ε-homocysteinyllysine (*N*ε-Hcy-Lys). Hcy-thiolactone is generated in an enzymatic error-editing reaction in protein biosynthesis when Hcy is erroneously selected in place of methionine by methionyl-tRNA synthetase. *N*-Hcy-protein is formed in a chemical reaction - called protein *N*-homocysteinylation - of Hcy-thiolactone with an ε-amino group of a protein lysine residue protein. *N*ε-Hcy-Lys is a product of proteolytic degradation of *N*-Hcy-protein. Each of those Hcy metabolites has been linked to atherothrombotic disease. In particular, protein *N*-homocysteinylation impairs or alters the protein's biological function, causes protein damage by a thiyl radical-mediated oxidation, and induces an auto-immune response. Dozens of protein targets for *N*-homocysteinylation, as well as specific *N*-Hcy lysine residues in individual proteins, have been identified *in vitro* and *in vivo*. *N*-Hcy-proteins undergo structural changes, which generate toxic amyloid-like aggregates. *N*-homocysteinylation of proteins involved in blood homeostasis (*e.g.*, fibrinogen) or tissue biogenesis (*e.g.*, collagen, elastin) leads to thrombosis or connective tissues abnormalities, respectively, frequently observed in severe hyperhomocysteinemia.

INV 21
Methionine metabolism in liver diseases

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Alcoholic liver disease is a major health care problem. Research in many laboratories, including ours has demonstrated that ethanol feeding impairs several of the multiple steps in methionine metabolism. Ethanol consumption has been reported to predominantly inhibit the activity of two vital cellular enzymes, methionine synthase and methyl adenosyltransferase involved in remethylating homocysteine and generating S-adenosylmethionine (SAM), respectively. By way of compensation in some species, ethanol increases the activity of the enzyme betaine homocysteine methyltransferase that catalyzes an alternate pathway in methionine metabolism and utilizes hepatic betaine to remethylate homocysteine to form methionine. All these alterations in enzyme activities by ethanol exposure principally result in a decrease in the vital liver metabolite, S-adenosylmethionine (SAM), and increase in two toxic metabolites, homocysteine and S-adenosylhomocysteine (SAH). The loss of SAM and an increase in SAH causes a decrease in the hepatocellular SAM:SAH ratios that leads to a plethora of detrimental functional consequences in the liver by particularly affecting many SAM-dependent methylation reactions. This defect ultimately

results in compromised liver function and progressive liver injury. Betaine administration, by remethylating homocysteine via the BHMT-catalyzed pathway, removes SAH, restores SAM levels and normalizes hepatic SAM:SAH ratios to maintain these and other essential methylation reactions. To conclude, betaine is a promising therapeutic agent in relieving the methylation and other defects associated with alcoholic abuse.

INV 23
Vitamin B-6 and folate affect the association between *MAT1A* variants and hypertension, stroke, and markers of DNA damage

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Background: The S-adenosylmethionine synthetase type 1 (MAT1A) gene encodes a key enzyme in one-carbon nutrient metabolism.
Aim: This study aimed to determine the association ofMAT1Avariants with homocysteine, DNA damage, and cardiovascular disease (CVD).
Methods: Eight variants of MAT1A were examined for associations with hypertension, stroke, CVD, homocysteine, and DNA damage in 1006 participants of the Boston Puerto Rican Health Study. Two variants were replicated in 1147 participants of the Nutrition, Aging, and Memory in Elders Study.
Results: Two variants and haplotypes were strongly associated with hypertension and stroke, independent of methylenetetrahydrofolate reductase genotypes. Homozygotes of theMAT1Ad18777A (rs3851059) allele had a significantly greater likelihood of stroke (OR= 4.30; P = 0.006), whereas 3U1510A (rs7087728) homozygotes had a lower likelihood of hypertension (OR=0.67; P = 0.022) and stroke (OR=0.35; P = 0.015). A similar trend of association was observed in a second elderly population. Furthermore, strong interactions betweenMAT1Agenotypes and vitamin B-6 status were found. Nonrisk allele 3U1510A carriers had a lower 8-hydroxydeoxyguanosine (8-OHdG) concentration— a biomarker of oxidative DNA damage—when plasma vitamin B-6 was high, whereas risk-allele 3U1510G homozygotes had higher 8-OHdG concentrations, regardless of vitamin B-6 status.
Discussion and Conclusions: MAT1Avariants were strongly associated with hypertension and stroke. Improving folate and vitamin B-6 status might decrease CVD risk of a subset of the population, depending on genotype. These findings suggest that impairments in methylation activity, independent of homocysteine, have an effect on CVD risk.

INV 24
Homocysteine and ADMA Influence Cerebrovascular and Myocardial Function through distinct mechanisms

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Background: The endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) is produced from proteins containing methylated arginine residues and is degraded by dimethylarginine dimethylaminohydrolase (DDAH). Homocysteine and ADMA are metabolically linked through the methionine cycle, and homocysteine inhibits the expression and activity of DDAH. Clinically, hyperhomocysteinemia is associated with both elevation of ADMA and adverse cerebral and cardiac vascular outcomes.
Aim: The goal of this study was to determine the mechanisms by which homocysteine and ADMA contribute to cerebrovascular dysfunction and myocardial ischemia-reperfusion (I/R) injury.
Methods: Heterozygous DDAH1-deficient ($DDAH1^{+/-}$) mice, DDAH1 transgenic (DDAH1 TG) mice and wild-type littermates (WT mice) were studied. Some DDAH1 TG and WT mice were fed a high methionine/low folate diet to induce moderate hyperhomocysteinemia. Endothelial function and cross-sectional area were measured in cerebral arterioles, and regional myocardial ischemia was produced by 30 minutes of myocardial ischemia followed by 120 minutes of reperfusion.
Results: Hyperhomocysteinemic mice developed endothelial dysfunction and hypertrophy of cerebral arterioles. Endothelial dysfunction correlated strongly with plasma levels of total homocysteine but not with plasma ADMA. Overexpression of DDAH1 protected from cerebrovascular hypertrophy but not from endothelial dysfunction in hyperhomocysteinemic mice. After myocardial I/R injury, larger infarcts were observed in $DDAH1^{+/-}$ mice than in WT mice. DDAH1 TG mice were protected from myocardial I/R damage, and hyperhomocysteinemia led to markedly increased myocardial injury both in DDAH1 TG and WT mice. Hyperhomocysteinemic mice had increased generation of superoxide during reperfusion, whereas $DDAH1^{+/-}$ mice had diminished cardiac levels of nitric oxide oxidation products.
Discussion and Conclusion: These findings suggest that homocysteine and ADMA produce adverse effects on cerebrovascular and myocardial function through distinct mechanisms. The observation that DDAH1 protects from cerebrovascular hypertrophy and myocardial injury in hyperhomocysteinemia suggests that ADMA may be a contributing factor (but not a primary cause) of vascular events in some cases of hyperhomocysteinemia.

INV 25
Betaine, both a methyl donor and an osmolyte

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Because of its behaviour as a solute, betaine (N,N,N-trimethylglycine) enhances the stability of proteins. Its concentrations in most tissues greatly exceed circulating concentrations, and are osmoregulated. This intracellular betaine has an important role in regulating cell volume. Its accumulation is both a major use of methyl groups, and a potential supply of them. The transfer of a methyl group from betaine to homocysteine is catalyzed by betaine homocysteine methyl transferase (BHMT), found in highest concentrations in human liver and renal cortex. BHMT is an abundant liver protein and its functions have not been fully elucidated. Its expression is osmoregulated and subject to control by hormones and betaine.
The supply of betaine is by the mitochondrial oxidation of choline and directly from the diet. Plasma concentrations are controlled and urinary excretion is minimal. Betaine deficiency has not been well defined, but could arise from poor diet with inadequate choline and betaine, from defective conversion of choline to betaine, or from an excessive loss of betaine. An abnormal betaine loss

in the urine is common in diabetes and related conditions, and in patients treated with fibrates. Sweat has also been found to have high betaine content. Betaine deficiency may be common in the metabolic syndrome. Betaine (often marketed as "TMG") is sold as a dietary supplement with strong claims about its health benefits. These claims are not supported by clinical studies. There is evidence that betaine supplements improve athletic performance, and long-term betaine supplementation of pigs improves the quality of pork (higher lean meat to fat ratio). Benefits in mouse and rat studies include less atherogenic plasma lipid profiles and decreased arterial plaque deposition. There is conflicting cross-sectional evidence in human populations. On the one hand high plasma betaine concentrations are associated with a favourable blood lipid profile and low BMI, on the other hand high plasma betaine concentrations have been associated with increased incidence of vascular events. In patients with elevated LDL cholesterol, urine betaine excretion is a major determinant of plasma homocysteine. In prospective studies both high and low plasma betaine concentrations predict heart failure and cardiovascular events, especially in diabetes. Both high and low betaine excretion predicts heart failure. The few studies of betaine supplementation demonstrate its safety, a lowering of plasma homocysteine and beneficial effects in alcoholic liver disease.
The connection between the known functions of betaine and lipid metabolism is obscure. Even if betaine deficiency is established to be a risk factor for a disease, there is still a need to demonstrate that betaine supplementation has a health benefit.

INV 26
Antiepileptic drugs and B-Vitamins: epidemiological and electrophysiological data

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Antiepileptic drugs (AED) are used for the treatment of epilepsy, psychiatric diseases and pain syndromes. Treatment with several AED seems to be associated with reduced folate and vitamin B12 serum levels, but respective studies were small. This prospective monocenter study aimed at testing which are associated with folate and vitamin B12 serum levels in a large population of 2730 AED-treated and 170 untreated patients with epilepsy and 200 healthy individuals. Treatment with carbamazepine, gabapentin, oxcarbazepine, phenytoin, primidone or valproate was associated with lower mean serum folate levels or with a higher frequency of folate levels below the reference range in comparison with all other patients, untreated patients or controls. Treatment with phenobarbital, pregabalin, primidone or topiramate was associated with lower vitamin B12 levels compared with the entire group of patients. Vitamin B12 serum levels were higher in patients treated with valproate compared with the entire group of patients, untreated patients and healthy controls. Folate or vitamin B12 levels below the reference range were associated with higher mean corpuscular volume (MCV) and higher homocysteine plasma levels. Vitamin substitution for three months in 141 patients with folate or vitamin B12 levels below the reference range yielded normal vitamin levels in 95% of the supplemented patients and reduced MCV and homocysteine plasma levels. In conclusion, treatment with most of the commonly used AED is associated with reduced folate or vitamin B12 serum levels and is a risk factor for hyperhomocysteinemia. Oral substitution is effective to restore vitamin, MCV and homocysteine levels.

INV 27
Fatty acid metabolism in choline and methionine deficiency in mice may influence progression to steatohepatitis but not fibrosis

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Background: We have previously shown that abnormalities of fatty acid metabolism may promote the development of steatohepatitis induced by a methionine and choline deficient diet (MCDD). However, its influence on progression to hepatic fibrosis is unknown.
Aims: To investigate the influence of fatty acid metabolism on the progression to fibrosis in fatty liver.
Methods: C57Bl6 mice (male, aged 14 weeks, n=7/group) were fed a MCDD, or choline deficient diet (CDD), to induce steatosis without inflammation or insulin resistance, or a supplemented control diet (CS) for 4 weeks and injected twice weekly with carbon tetrachloride (CCl_4, 0.3 µl/g in olive oil (OO), 1:3 v/v) or vehicle (OO) to induce hepatic fibrosis.
Results: Liver TG pools were similarly elevated in both CDD and MCDD mice (~2 fold), and were unaffected by CCl_4. MCDD, but not CDD, caused liver inflammation (increased hepatic neutrophils) and increased mRNA levels of the pro-inflammatory cytokines TNFa and IL-1b. MCDD, but not CDD, also increased mRNA levels of the pro-fibrotic cytokine TGFb and increased hepatic stellate cell activation (alpha smooth muscle actin (aSMA) mRNA levels and staining). CCl_4 further increased aSMA staining in MCDD mice alone, but there was no additional effect of CCl_4 on gene expression in either group. Picosirius red staining confirmed CCl_4 induced fibrosis, but there was no increase in either model of fatty liver.
Discussion and Conclusion: These findings suggest that whilst abnormalities of hepatic fatty acid metabolism may promote the development of steatohepatitis, they do not increase susceptibility to liver fibrosis. Additional factors such as reduced leptin levels or increased matrix metalloproteinase activity may act to limit progression.

INV 28
Folate Intake Modulates Carcinogenesis: Mechanisms and Consequences

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Epidemiologic evidence generally indicates that an abundant intake of foodstuffs rich in folate conveys protection against the development of colorectal cancer and perhaps some other common cancers as well. Preclinical models substantiate that the relationship is a genuinely causal one. Emerging data suggests that availability of the other 'one-carbon vitamins'-B2, B6, and B12 - may also impact on the phenomenon in a synergistic fashion. It is entirely possible that the cancer preventive effects of folate and other B-vitamins are mediated through several cellular pathways, but recent observations most strongly implicate the Wnt signaling pathway.
However, the issue is rather complex because some observations in animal and human studies demonstrate that an overly abundant intake of folate among those who harbor existing foci of neoplasia

might instead produce a paradoxical promotion of tumorigenesis. The pharmaceutical form of the vitamin, folic acid, might affect the process in a manner that is distinct from natural forms of the vitamin, although this remains a speculative concept. We should not allow the complex nature of this relationship to compel us to ignore it, as understanding its true nature will greatly facilitate our ability to construct intelligent, effective, and safe strategies for the prevention of birth defects and cancer.

INV 29
Haptocorrin a teaser for researchers and for routine measurement of cobalamin

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Haptocorrin (HC) is the youngest of the soluble cobalamin-binding proteins and has evolved after diversion of the bony fish. It is expressed in most but not all mammals, notably not in mice.
In humans, HC is present in plasma and in most extracellular fluids, such as saliva and milk.
In plasma, HC carries most of the circulating cobalamin and in addition a comparable amount of degradation products (analogues). Interestingly, analogues accumulate on HC during foetal life, but no analogues are present on HC in human milk.
The level of plasma cobalamin is driven by the level of HC rather than by holoTC, the active part of plasma cobalamin. Decreased level of HC, and thereby plasma cobalamin, is observed during pregnancy, while a moderate increase in HC is related to liver diseases and certain forms of cancer. Extreme levels are seen in patients with fibrolamellar hepatocellular carcinoma, where HC may serve as a tumour marker.
High levels of HC interferes with measurement of cobalamin resulting in spurious low or high cobalamin levels depending on the analytical method employed. This problem can be solved by removal of unsaturated HC, a pre-treatment that is mandatory in order to obtain a correct estimate of cobalamin in human milk.
The function of HC remains unsolved. Life goes on without its presence. Mice do not express HC but express transcobalamin where humans have HC. The presence of HC in milk combined with its ability to bind analogues led to a suggested function in modifying the intestinal microbiota of the newborn, but recent studies could not confirm such a function.
In conclusion, haptocorrin remains a challenge. Researchers need to clarify its function and in the routine lab, it may interfere with measurement of cobalamin.

INV 30
Intracellular cobalamin and methylation markers in patients with type 2 diabetes

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Background: Vitamin B12 deficiency might be related to peripheral neuropathy (PNP) in patients with type 2 diabetes. Elevated concentration of methylmalonic acid (MMA) and total homocysteine (tHcy) are common in PNP patients. The current study aimed at studying mechanisms responsible for altered cobalamin homeostasis in diabetics and the relationship between cobalamin status and hypomethylation as one important mechanism in PNP.
Methods: The study included 92 patients type 2 diabetes and different degrees of PNP and 72 controls. We measured cobalamin markers (tHcy, MMA, total cobalamin, holoTC), RBC-cobalamin, blood methylation markers [S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH)], and diabetic parameters (HbA1C, glucose). We also investigated the grad of PNP.
Results: Diabetic patients had compared with controls significantly higher plasma SAH (15.2 vs. 11.8 nmol/L: p=0.001) and lower SAM/SAH ratio (9.0 vs. 8.2). In contrast to serum MMA that was higher in diabetics (256 vs. 207 nmol/L: p=0.01), concentrations of total cobalamin and holoTC did not differ significantly between the groups. However, RBC-cobalamin was significantly lower in diabetics compared to the controls (median 230 vs. 260 pmol/L). An inverse correlation between MMA and RBC-cobalamin was found (r=10.40, p<0.001). SAM/SAH ratio correlated positively to RBC-cobalamin and negatively to serum MMA, suggesting that low cellular cobalamin status is associated with lower methyl group synthesis. Cobalamin markers were related to metformine usage. Serum holoTC increased with worsening of PNP symptoms; possibly due to impaired cellular uptake of holoTC. Two mechanisms of cobalamin resistance will be discussed.
Conclusion: Diabetic patients showed an impaired cobalamin status despite normal holoTC and total cobalamin in serum. Altered cobalamin metabolism may play a role on PNP.

INV 31
Hydrogen Sulfide and Oxygen Sensing

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Hydrogen sulfide (H_2S) has been shown to have numerous effects on biological systems and it has been classified along with NO and CO as a "gasotransmitter". Recent evidence suggests that H_2S is the elusive oxygen "sensor" that directly couples tissue Po_2 to appropriate physiological responses. In the cardiovascular system this enables pulmonary and systemic blood vessels to directly match perfusion to either ventilation or metabolism, respectively, and it is the mechanism for chemoreceptor transduction of blood and environmental Po_2. This O_2-sensing mechanism consists of a simple balance between H_2S production in the cytoplasm and its Po_2-dependent oxidation by the mitochondria. A number of observations support this hypothesis; 1) the effects of hypoxia and H_2S on a variety of tissues including vascular and non-vascular smooth muscle and chemoreceptor cells are virtually identical, 2) H_2S "donors" augment hypoxic responses and inhibition of H_2S production inhibits hypoxic responses, 3) deletion of cystathionine γ-lyase, an H_2S-producing enzyme blunts hypoxic hyperventilation, 4) inhibitory effects of H_2S on chemoreceptor potassium channels is consistent with hypoxic activation, and 5) the transition from O_2-dependent H_2S consumption to H_2S production occurs precisely over the physiological Po_2 transition from normoxia to hypoxia. H_2S-mediated O_2 sensing is also supported by anecdotal evidence including the reciprocal relationship between H_2S and O_2 in the environment, the origin of eukaryotic cells from the combination of sulfide reducing Archaea and sulfide oxidizing α-protobacteria and by studies that show H_2S increases O_2 consumption in many tissues. Support: NSF IBN0235223, IOS0641436 and IOS1051627.

INV 32
Mechanisms of H_2S signalling in hemodialysis

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Hydrogen sulfide, H_2S, is a gaseous compound involved in a number of biological responses, e.g. blood pressure, vascular function, energy metabolism. In particular, H_2S is able to lower blood pressure, to protect from injury in models of ischemia-reperfusion, and to induce a hypometabolic state. In chronic kidney disease (CKD), low plasma hydrogen sulfide levels have been established in humans and in animal models. The enzymes involved in its production are cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopyruvate sulfurtransferase. The mechanisms for H_2S decrease in CKD are related to the reduced gene expression (demonstrated in uremic patient blood cells) and decreased protein levels (in tissues such as liver, kidney, brain in a chronic kidney disease rat model). It has been shown that alterations in this pathway complicate the uremic state and are linked to chronic kidney disease progression. It remains to be established if low H_2S is causally linked to CKD progression and if interventions aimed to restore the status quo ante are able to modify this picture.

INV 33
Riboflavin as a determinant of iron status

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Background: Riboflavin, in the form of derivatives flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) is essential for many redox reactions in intermediary metabolism. Additionally, experimental evidence suggests that the importance to oxidation/reduction reactions may extend to aspects of iron handling. The relevance of this to human populations is uncertain.
Aim: To investigate the effect on haematological status of improving riboflavin status in young women in the United Kingdom.
Methods: A randomised placebo controlled trial was conducted in women aged 19 to 25 years with biochemical evidence of poor riboflavin status. Women were randomised to receive 2mg or 4mg riboflavin, or a placebo, daily, for 8 weeks. Effects of the intervention on measures of iron status and iron bioavailability were determined.
Results: There was a dose-response improvement in riboflavin status in response to the supplement. Improvement in riboflavin status elicited an increase in hemoglobin and red blood cell number, being greatest in those women with poorer riboflavin status at the outset. Women in the lowest tertile for riboflavin status at baseline showed a significantly greater improvement in hemoglobin and red blood cell number than women in the highest tertile ($P<0.01$) and those in the middle tertile ($P<0.05$). There was no change in dietary iron or riboflavin during the study. Results could not be explained by an improved iron bioavailability but there was a suggestion that the supplement might have enhanced mobilisation of ferritin iron stores.
Discussion and Conclusion: Improving riboflavin status can have a beneficial effect of measures of iron status, independent of dietary iron.

INV 34
Advances In Cobalamin Research: Targeting The Transcobalamin Receptor TCblR/CD320

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Background: Cellular uptake of cobalamin (Cbl) is mediated by TCblR, a membrane receptor for transcobalamin (TC)-bound Cbl. Identification of the gene encoding this protein asCD320has enabled us to produce the gene knockout mouse and characterize the protein.
Aim: Characterize the CD320knockout mouse; Identify structural and functional domains of the receptor; study the expression of TCblR in various tumor cell lines and use monoclonal antibodies for targeted delivery of toxins and drugs to cancer cells
Methods: The CD320knockout C57B1/6N mouse was generated from 129P2 Ola ES cells. Monoclonal antibodies to the extracellular domain of TCblR were produced using recombinant TCblR expressed in HEK293 cells
Results: The CD320knockout is not lethal and the mice develop and reproduce normally. While serum, liver and kidney B12 is normal, Selective depletion of Cbl is seen primarily in the CNS and less so in bone marrow and spleen. The Peripheral nerve and spinal cord show myelin loss that is a hallmark of human B12 deficiency. The cell cycle associated receptor expression is up regulated in some cancers and monoclonal antibodies to the receptor, are effectively internalized by cells. The two LDLR-A domains in the extracellular region and the cytoplasmic tail are critical determinants of TC-Cbl binding and internalization.
Discussion and Conclusions: The CD320knockout mouse with the characteristic demyelination is an excellent model to identify pathways and genes involved in metabolic disorders contributing to the demyelinating pathology and monoclonal antibodies to the external domain of the receptor provide a vehicle to deliver cytoxic drugs and toxins to cancer cells.

INV 35
Genetic and nutritional variation in one-carbon metabolism influences reproductive outcomes

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Homocysteine remethylation is an important metabolic reaction because it generates methionine and S-adenosylmethionine for methylation reactions. It also serves to limit accumulation of homocysteine, a potentially toxic amino acid. The major homocysteine remethylation pathway uses 5-methyltetrahydrofolate (synthesized by methylenetetrahydrofolate reductase (MTHFR)). An alternate pathway uses betaine, derived from choline, as a methyl donor. We have been studying reproductive outcomes (including resorptions, embryonic delays, and heart defects) in mice with a deficiency of MTHFR (Mthfr+/-) and in mice fed diets that are low in folate (7-fold lower) or choline (8-fold lower), compared to the recommended diets for rodents. We have also examined outcomes in mice fed high folate diets (20x and 10x higher than recommended) since some populations are consuming higher levels of folic acid due to food fortification or vitamin supplementation. Our findings suggest that diets low in methyl donors, or high in folate, lead to adverse reproductive outcomes. In pursuing some of the mechanisms that

lead to the complications in methyl deficiency states, we have identified changes in some inflammatory mediators including ApoAI, PPARα, IFNγ and IL-10. Details of these experiments will be presented.

INV 36
Normal prions as a new target of cobalamin deficiency in rat CNS

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It is known that cobalamin (Cbl) deficiency damages myelin by increasing tumour necrosis factor(TNF)-α and decreasing epidermal growth factor(EGF) levels in rat central (CNS) and peripheral nervous system (PNS), and that TNF-α and EGF regulate normal prion protein (PrP^C) expression. We investigated whether: a) the octapeptide repeat (OR)-region of PrP^C (which is claimed to be myelinotrophic) is involved in the pathogenesis of rat Cbl-deficient (Cbl-D) neuropathy; and b) Cbl deficiency modifies PrP^C levels of spinal cord (SC) and PNS in the rat. We intracerebroventricularly administered antibodies against the OR-region (OR-Abs) to Cbl-D rats to prevent SC and PNS myelin damage and maximum nerve conduction velocity (MNCV) abnormalities, and PrP^Cs to otherwise normal (ON) rats to reproduce Cbl-D-like lesions. The OR-Abs (but not when inactivated) normalized myelin ultrastructure and TNF-α levels in the SC and peripheral nerves, and MNCV values of Cbl-D rats. PrP^C levels had increased in SC and peripheral nerves of Cbl-D rats by the time myelin lesions appeared. These increases were mediated by excess TNF-α. There were no changes in hepatic PrP^C levels of Cbl-D rats. Cbl deficiency greatly reduced SC PrP^C-mRNA levels, which were subsequently increased by Cbl and EGF, which proved to be effective in preventing the typical Cbl-D lesions. The SC and PNS of ON-, PrP^C-treated rats showed typical Cbl-D lesions, significantly increased TNF-α levels, and significantly decreased MNCV values. Therefore: a) the number of OR regions in rat CNS and PNS seem to be "buffered" by Cbl; b) Cbl deficiency causes a vicious circle between TNF-α and PrP^Cs in rat CNS and PNS; and c) new PrP^C synthesis is a common effect of different myelinotrophic agents in rat SC.

INV 37
Cellular choline metabolism is related to endoplasmic reticulum stress in vascular and metabolic diseases

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Choline is interrelated to folate in one-carbon metabolism serving as methyl-donors for homocysteine re-methylation. Polymorphisms in choline and folate pathways require folate or choline to balance re-methylation through the non-affected pathway. Organ specific PEMT activity determines the pattern of convertible and non-convertible PC/PS/PE iso-species and the ER stress response in diabesity. Availability of choline or disturbed incorporation in phospholipids are critical determinants of hyperhomocysteinemia. Disruption of the choline-phospholipid axis leads to the formation of acyl-glycerols and affects lipid storage as a hallmark of fatty liver diseases. A fructose-fed rat model revealed that the fenofibrate induced increase in homocysteine may reflect a "choline stealing" phenomenon due to channeling of choline towards PC metabolism rather than methyl-group donation. In response to HDL3 macrophages up-regulate their "convertible" PC species 36:1, 36:4 and 38:4 and "non-convertible" PC 36:5 and 38:6 in parallel with the down-regulation of all other "non-convertible" PC-species. We tested the in vivo response of blood monocytes to an oral fat load. Control and metabolic syndrome patients showed either an increase or unchanged levels of all "convertible" PC species. Among the "non-convertible" PC species only 34:0 was upregulated in control subjects, while most PC species were stable or showed a downregulation (32:0, 36:3). Under these conditions the integration of ER-stress (metabolic overload, cancer) oxidative stress (ROS, glutathione) and the chronic inflammatory response (CRP, NFκB) in the elderly is critical to the pathogenesis of vascular- and metabolic disease as well as cancer.

INV 38
Renal function determines response to B-vitamin therapy for homocysteine

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Background: B-vitamin therapy to lower homocysteine has been thought to be ineffective in reducing cardiovascular risk. However, interpretation of clinical trial results has not adequately taken account of the role of renal function.
Aim: To interpret clinical trial results in the light of new evidence that B-vitamin therapy is harmful in patients with impaired renal function.
Methods: Contrasting beneficial effect of B-vitamin therapy in the VISP efficacy analysis, from which patients with renal impairment were excluded, with those of the DIVINe study, in patients with diabetic nephropathy.
Results: In the VISP efficacy analysis, from which patients with GFR in the lowest 10% (<50).
Discussion and Conclusion: Increased production of asymmetric dimethylarginine, an antagonist of NO, and accumulation of cyanide from cyanocobalamin (leading to consumption of hydrogen sulfide (an endothelium-derived relaxing factor) may explain the harmful effects of B-vitamins in renal failure. To lower tHcy in patients with renal failure it will be better to use methylcobalamin and more intensive dialysis; thiols such as mesna are also being studied in this group.

INV 39
Choline - containing phospholipids on brain functional pathways

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Choline (Ch) is involved in relevant neurochemical processes. It is the precursor and metabolite of acetylcholine (ACh). It plays a role in single-carbon metabolism and is an essential component of different membrane phospholipids (PLs). These PLs are structural components of cell membranes, and involved in intraneuronal signal transduction. An increased ACh release was found after Ch treatment in rat corpus striatum slices. An in vivo proton magnetic resonance study has analyzed Ch ingestion effect. This work which represents the first

non invasive study for exploringin vivohuman brain neurochemistry showed the transfer of an oral Ch load in the brain of normal volunteers. These results were not confirmed by otherin vivostudies. Cellular membranes breakdown is suggested as a feature of neurodegeneration in acute (stroke) and chronic (Alzheimer's and vascular dementias) brain disorders. The effects of exogenous CCPLs on different brain areas were largely studied. Our group has assessed the influence of treatment with the CCPL, choline alphoscerate (GPC) on brain cholinergic neurotransmission markers in an animal model of brain vascular injury. A neuroprotective effect of GPC alone or in association with acetylcholinesterase inhibitor, galantamine was found. These results suggest that GPC could stimulate the expression of vesicular ACh transporter and Ch transporter primarily in areas involved in cognitive processes. These cholinergic markers could represent an appropriate mean to investigate brain cholinergic pathways. In the lack of novel therapeutic strategies, safe compounds developed since a long time such as the CCPLs could have still a place in pharmacotherapy and would merit to be investigated by new clinical studies.

INV 40
Choline as a treatment for fetal alcohol spectrum disorders

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Background: Children exposed to alcohol prenatally may suffer from a range of physical, neurological, and behavioral alterations, referred to as fetal alcohol spectrum disorders (FASD). Identification of effective treatments for FASD is critical.
Aim: Using an animal model, we have been investigating the ability of choline, an essential nutrient, to reduce the severity of FASD.
Methods: Rats were exposed to alcohol during development and the effects of choline administration on physical, behavioral, and neural development were evaluated.
Results: Choline supplementation during prenatal alcohol exposure reduces alcohol-related birth weight deficits and alterations in motor and cognitive development. However, even if administered postnatally, choline reduces the severity of alcohol-induced deficits on cognitive tasks that depend on the functional integrity of the hippocampus and/or prefrontal cortex. Moreover, choline attenuates alcohol-related alterations in hippocampal cholinergic receptors, and alters alcohol's effects on global DNA methylation.
Discussion and Conclusion: Choline supplementation can mitigate alcohol's teratogenic effects on behavioral development, likely acting, in part, by altering cholinergic functioning and epigenetic mechanisms. These data have important implications for individuals with FASD and suggest that dietary interventions may reduce the severity of some prenatal alcohol effects, even when administered postnatally. Supported by AA12446 & 014811.

INV 41
Disturbance of thiamine metabolism in diabetes and the development and treatment of diabetic vascular complications

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Background: Thiamine (vitamin B_1) is an essential micronutrient required for health. Converted to thiamine pyrophosphate, it is the cofactor for enzymes pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and transketolase.
Aim: Studies have been performed over the past 10 years to characterize the mishandling of thiamine in experimental and clinical diabetes and evaluate the effect of thiamine supplements on the development of vascular complications.
Methods: Experimental studies employed cell cultures in low and high glucose concentrations and streptozotocin-induced diabetic rats with maintenance insulin therapy to moderate diabetes. Clinical studies have involved cross-sectional observational studies and randomized, double-blind intervention trials.
Results: There is increased renal clearance of thiamine in experimental and clinical diabetes linked to decreased re-uptake of thiamine in the proximal tubules in diabetes associated with decreased expression of thiamine transporter proteins in the proximal tubular epithelium. Similar impaired re-uptake and retention of thiamine in diabetes is present in the retina and peripheral nerve. It is improved but not corrected by intensive glycaemic control. Thiamine supplementation prevented the development of diabetic nephropathy in experimental diabetes and reversed early stage diabetic nephropathy in patients with type 2 diabetes in two clinical intervention studies.
Discussion and Conclusion: Tissue-specific mishandling of thiamine in diabetes occurs at sites of development of vascular complications and may predispose to vascular disease and thiamine supplements prevent and reverse early stage vascular complications of diabetes.

INV 42
Mechanisms of Neurological complications in HHCY

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The basis for association of elevated plasma total homocysteine with neurological dysfunction has been explored in a wide variety of cellular and animal models. These studies demonstrate that experimental exposure to homocysteine and/or perturbation of homocysteine metabolism and its associated pathways can induce a remarkable range of molecular, cellular and neurological abnormalities. These abnormalities include changes in processes that may contribute to chronic neurodegenerative and cerebrovascular disease (vascular dysfunction, inflammation and oxidative stress, abnormal NO signaling, etc.), as well as some that are specifically involved in Alzheimer's disease (such as enhanced amyloidogenesis and tau phosphorylation). While most studies report on the harmful effects of exposure, few studies have indicated that damage can be reversed. Nevertheless, the diversity of hypothetical mechanisms provides a similar diversity of non-exclusive, potential therapeutic targets. However, the validity and generalizability of the experimental models and their hypothetical mechanisms to human neurological dysfunction remains uncertain. Part of the problem lies in defining the nature of the relevant exposure. Elevated homocysteine in humans is a non-specific biomarker for perturbed metabolism which can be caused by discrete or overlapping nutritional deficiencies, genetic abnormalities, or endocrine and pathological conditions. Although homocysteine may be intrinsically cytotoxic, different metabolic disturbances upstream of hyperhomocysteinemia can have different neurological effects. Thus different models of experimental homocysteinemia can lead to different neurological outcomes. Furthermore, the generalizability of findings and postulated mechanisms in these models may be constrained by the

method of inducing homocysteinemia; the timing and duration of exposure; age, sex, genetic background, and co-existing physiological or pathological phenotypes. In this respect, the experimental data mirrors the inconsistent associations observed in the epidemiological literature between homocysteine, folate and vitamin B12 with different cognitive domains and neuropatholgical outcomes. Indeed the inconsistent results from randomized controlled clinical trials of B-vitamin therapy for homocysteine lowering and neuroprotection, show that homocysteine lowering alone may be insufficient to yield therapeutic benefit, leaving open the question of homocysteine's role in neurological disease. Better differentiation of the metabolic and pathologic mechanisms linking homocysteine to neurological dysfunctions in humans will be important to improve future interventions.

INV 43
Homocysteine mediated decrease in bone blood flow and remodeling: Role of Folic Acid

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Background: Homocysteine (Hcy) is a non-essential sulfur-containing amino acid. Deficiency in cystathionine-β-synthase, (CBS) enzyme lead to increased serum concentrations of Hcy, which is known as hyperhomocysteinemia (HHcy), is associated with bone disorders. Although, Hcy accumulates collagen in bone and contribute to decrease in bone strength. The mechanism of Hcy induced bone disorders and remodeling is unclear.
Material and Methods: Wild type (WT) and cystathionine-β-synthase heterozygous (CBS+/–, genetically HHcy) mice were used in this study and supplemented with or without FA (300 mg/kg, Hcy reducing agent) in drinking water for 6 weeks. The tibial bone blood flow was measured by laser Doppler and ultrasonic flow probe method. The tibial bone density (BD) was assessed by dual energy X-ray absorptiometry. The bone homogenates were analyzed for oxidative stress, NOX-4 as oxidative marker and thioredoxin-1 (Trx-1) as anti-oxidant marker, bone remodeling (MMP-9) and bio-availability of nitric oxide (eNOS/iNOS/NO) by Western blot method.
Results: The results suggested that there was decrease in tibial blood flow in CBS+/– mice. The BD was also reduced in CBS+/– mice. There was an increase in NOX-4, iNOS, MMP-9 protein as well as MMP-9 activity in CBS+/– mice and decrease in Trx-1, eNOS protein levels, in part by decreasing NO bio-availability in CBS+/– mice. Interestingly, these effects were ameliorated by FA.
Conclusions: The results of the present study suggest that FA supplementation may have therapeutic potential against genetically HHcy induced bone loss

INV 44
Diet as source of methyl donors nutrients: from raw to "ready-to-eat" foods

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By definition, a methyl donor is a substance capable of donating a methyl group. Dietary methyl groups derive from foods that contain methionine, one-carbon units and choline or betaine. Transmethylation metabolic pathways closely interconnect choline, methionine, methyltetrahydrofolate (methyl-THF) and vitamins B-6 and B-12. Many important biochemical processes and healthy/unhealthy situations may rely on methylation capacity, although the research in this area is still emerging. The pathways intersect at the formation of methionine from homocysteine. Perturbing the metabolism of one of these pathways results in compensatory changes in the others. There may also be of value dietary measures that integrate all these nutrients, "methylation capacity". Different scenarios are approached at the presentation.
To promote methyl donor nutrients intake from food sources is a healthy strategy taking into account that chronic excessive intakes through fortified food products or supplements may be harmful in some populations groups. Recently, an expansion of the consumption of ready-to-eat foods constitutes an important portion of the diet resulting in an increasing concern about their nutrient density. Accordingly, the nutritional composition should be well known to estimate its contribution. This presentation confirms the increased consumption of ready-to-eat products in Europe, showing the importance of giving data about their nutritional composition. In addition, the "worldwide map of methyl-nutrients fortification" will be analyzed. An important question also to be addressed is how much is too much? Finally, the "risk/benefit" dilemma is also considered in the "new" methyl-donors fortified world.

INV 45
Molecular mechanisms of homocysteine effects on bone cells

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Elevated homocysteine (Hcys) serum levels are reported to be a risk factor for several chronic disorders such as cardiovascular disease, atherosclerosis, chronic renal failure, diabetes, and metabolic syndrome. Moreover, hyperhomo-cysteinemia is known to affect bone development and homeostasis. Biochemically, Hcys inhibits lysyl oxidase (LOX), the enzyme responsible for collagen cross-linking and affects, thereby, bone quality, amongst other effects.
The aim of the present study was to elucidate whether Hcys affects proliferation and differentiation of osteoblastic cells as well as their matrix synthesis.
MC3T3-E1 preosteoblastic cells were treated with Hcys up to 21 days. Gene expression was analyzed by RT-PCR, gene chips and immune-blots. CpG methylation of Fasand Loxgene promoters was determined. Changes in collagen cross-linking were measured by Fourier Transform Infra Red spectroscopy.
The results indicate that Hcys affects proliferation of MC3T3-E1 cells and differentiation by up-regulation of Run x2 and genes modulating RUNX2 function. Most interestingly, Hcys down regulated expression of LOX by CpG methylation of its promoter DNA via interleukin-6 that up regulated DNA-(cytosine-5)-methyltransferase-1, the enzyme responsible for epigenetic DNA methylation.
Down-regulation of LOX resulted in changes of collagen cross-linking and partial denaturation of collagen triple helices, as expected, by unlocking the RGD-motif. This resulted in up-regulation of SAA3, an acute phase protein related to chronic pathologies, and MMP13, a protease known to induce bone resorption.
The data of the present study suggest that hyperhomocysteinemia affects bone quality by down-regulation LOX and the development of degenerative skeletal diseases by up-regulation of SAA3.

INV 46
Exploring the genetics of choline metabolism: Unexpected observations

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Choline is known to be important for methyl donation, membrane synthesis and for neurotransmission. Humans eating diets low in this nutrient develop liver and muscle dysfunction. In addition, during pregnancy choline is important for normal fetal development. While conducting studies in humans it became apparent that polymorphisms in genes of choline and folate metabolism modulated the dietary requirements for choline. For this reason we conducted studies to help us to understand the importance of these genes. Phosphatidylethanolamine-N-methyltransferase (PEMT) is induced by estrogen, and for this reason young women have a decreased dietary need for choline because they use this enzyme to endogenously produce phosphatidylcholine, a source of choline. We identified a very common single nucleotide polymorphism (SNP) in this gene that abrogates estrogen induction of the gene and thereby increases dietary requirements for choline. Pemtknockout mice have diminished docosahexaenoic acid in membrane phosphatidylcholine, have excessS-adenosylmethionine and have increased DNA methylation with increased neurogenesis. Choline dehydrogenase (CHDH) forms betaine from choline. Chdhknockout mice have mitochondrial dysfunction, decreased ATP synthesis, and sperm motility abnormalities. Humans with a functional SNP inCHDHalso have abnormal mitochondrial and low ATP in sperm. Betaine homocysteine methyltransferase (BHMT) uses the methyl group of betaine to form methionine from homocysteine. Bhmtknockout mice have fatty liver, decreased fat mass, increased glucose oxidation, increased insulin sensitivity, and develop liver cancers. These studies in mice suggest that choline has a role in energy metabolism that is unexpected.
This work was support by grants from the NIH (DK55865 and DK36530)

ORAL PRESENTATIONS

Vitamin B12 physiology

WS1 01
Haptocorrin – The key to vitamin B12 dependent tumour targeting?

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Background: In the human blood stream vitamin B12 (Cbl) is transported by Haptocorrin (HC) and transcobalamin (TC). In contrast to TC, the physiological function of HC is largely unknown. Recently, we could show that a radio-labelled, HC-selective Cbl-derivative (Tc-99m-Cbl(PAMA4) homes for tumours.
Aim: To explore the potential of HC-binding drugs as tools for tumour targeting and treatment.
Methods: We established two HC expressing mouse models of human cancer. Mice were injected with Tc-99m-Cbl(PAMA4) and imaged with SPECT/CT. Accumulation in tumors and organs was quantified by *ex vivo* biodistribution studies.
We established the recombinant expression and purification of HC (rhHC) in HEK293 cells. The structure of rhHC was solved with molecular replacement and refined to a resolution of 2.6Å.
Binding of Tc-99m-Cbl(PAMA4) was monitored by spectral analysis and gel shift assays.
Results: rhHC was expressed and purified to >99 % purity. Crystallisation lead to structure determination of rhHC in complex with Cbl.
Biodistribution studies showed specific accumulation of Tc-99m-Cbl(PAMA4) in mouse models of human cancer. Compared to reported Cbl-derivatives, uptake of Tc-99m-Cbl(PAMA4) in non-targeted organs and tissue was low.
Discussion and Conclusion: We demonstrated that HC-selective ligands specifically accumulate in HC-expressing tumours. In addition, we identified structural features of HC that account for ligand selectivity and can be exploited for future drug design.

WS1 02
Clinical and biochemical assessment of high serum vitamin B12 levels

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Introduction: Measurement of serum cobalamin (Cbl) is routinely used to assess suspected Cbl deficiency. Surprisingly, 15% of all samples analysed for serum Cbl show values above the reference range of 200-600 pmol/L.
Aim: We hypothesized that increased Cbl levels are caused by alterations in the circulating Cbl binding proteins haptocorrin (HC) and/or transcobalamin (TC), and that such changes may be of clinical importance.
Materials and methods: We collected 834 blood samples from hospital treated patients with serum Cbl levels: <200, 200-600, 601-1000 and >1000 pmol/L. In-house ELISAs were used for measurement of HC and TC. Data on diagnoses and medication was obtained from the electronic patient medical chart in the Region of Central Denmark and the Aarhus University Prescription Database.
Results: In 38% of patients, high Cbl levels were attributable to Cbl supplementation. Among non-treated patients higher HC levels were associated with higher Cbl levels, while TC levels were within reference range for all Cbl levels. Significantly higher proportions were found for diagnoses of alcoholism, liver disease, cancer and non-malignant bronchopulmonary disease when comparing non-treated patients with both high HC and high Cbl levels and patients with high HC levels and normal/low Cbl levels.
Conclusion: Unexplained high Cbl levels are mostly due to high HC levels and several diagnoses may be important to consider when patients show high Cbl levels.

WS1 03
Efficacy of treatment options in vitamin B12 deficiency

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Method: Informed consenting vegetarian subjects (n=122), not taking vitamin supplements, with vitamin B12 deficiency were recruited

for the study. Each participant had blood taken for a full blood count, twenty-test biochemistry screen, and serum vitamin B12 and homocysteine levels. A validated wellbeing questionnaire was also administered.
Participants were assigned to one of six age and gender matched groups:
Controls who received a placebo;
Low-dose tablets (2 ug vitamin B12/day)
High dose tablets (50 ug vitamin B12 once/week)
Vitamin B12 fortified Soy beverage (containing 2 ug vitamin B12/day)
Meat substitute containing 0.6 or 0.8 ug vitamin B12/day)
IM injection (100 ug vitamin B12/month)
Participants received treatment for three months. After a four -week equilibration period, participants were again tested for serum vitamin B12 and homocysteine levels and the Wellbeing questionnaire was again administered.
Results:

	Control	IM Injection	Lodose tablet	Hi dose tablet	Fortified Meat substitute	Fortified soymilk alternative
Dose B12 received	Nil	100 ug/ month	2 ug/ day	50 ug/ week	0.8 ug/ day	2 ug/day
Total B12/month	Nil	100	60	50	24	60
Mean % increase in serum B12	-2.3	+21	+18.7	+12.7	+5.7	+31.7
P value	0.18	0.0009	0.03	0.003	0.24	<0.0001
Mean % decrease in homocysteine	-4.2	-3.6	-3.8	-11.0	-5.6	-16.9
P value	0.07	0.11	0.008	0.003	0.09	0.0007
Initial wellness score	84	79	87	80	78	80
Final wellness score	88	83	95	86	81	86
P value	0.23	0.11	0.009	0.0002	0.18	0.01

Conclusions: Vitamin B12 fortified soy milk alternative is effective in treatment of vitamin B12 deficiency.
Daily low-dose tablets are more effective than weekly high-dose tablets. There is an inverse relationship between vitamin B12 levels and homocysteine (body reserves). People 'feel better' after treatment with vitamin B12.

WS1 04
Repression signaling pathways of *MDR-1* gene : A link between vitamin B12 and chemotherapy?

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Background: A key factor of chemioresistance is an increased expression of *MDR-1* gene, partly controlled by cellular methylation reactions. Until now, the physiology of these reactions is not clearly known. The main intracellular metabolic pathway, generating methyl donors, is the methionine cycle, the activity of which is strongly depending on B-group vitamins (B12, B9). Thus, *MDR-1* gene expression may be controlled by the activity of the methionine cycle and consequently presence of these vitamins.
Aim: The aim of this study is to determine if, and to elucidate how, the methionine cycle influences the *MDR-1* gene expression.
Methods: Chromatography, pharmacotoxicology, gene and protein expression studies have been used on the human hepatocarcinoma cell line HepG2.
Results: We showed that cobalamin induces a dose-dependent repression of *MDR-1* gene expression. However, this is not linked to changes in the methylation profile of its promoter but in methylation of membrane phospholipids. Furthermore, we showed that *MDR-1* cobalamin-induced repression was associated with phospholipase D activation, Akt phosphorylation, and Cox-2 co-repression.
Discussion/Conclusion: We show for the first time a link between a cobalamin-sensitive activity of the methionine cycle and repression of *MDR-1* gene. This includes a pathway involving phospholipase D and Akt activation as well as Cox-2 repression. This also suggests that patients' vitamin status should be taken into account as it might influence their sensitivity to chemotherapy. Furthermore, the involved intracellular pathways might be potential targets in order to potentialize current, or develop new, anticancerous therapies.

WS1 05
Responsiveness of urinary methylmalonic acid and other B12 biomarkers to oral supplements of vitamin B12

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Background: There is a consensus of opinion that plasma B12 is lacking as a marker of vitamin B12 status. Although alternative biomarkers have received attention urinary methylmalonic acid (uMMA) has been neglected.
Aim: A study was conducted to determine the responsiveness of uMMA and conventional biomarkers of B12 status, to graded oral supplements of vitamin B12, in a healthy elderly population.
Methods: A double-blind, placebo-controlled, randomized trial was carried out in 100 men and women aged 65 to 86 years, with generally poor B12 status. Treatment groups were placebo, 10µg, 100µg or 500 µg B12 daily for 8 weeks. Fasted urine samples were collected at baseline and every two weeks, for measurement of uMMA and creatinine, and blood samples were collected at baseline and after 56 days, for measurement of plasma B12 and MMA and serum holotranscobalamin (holoTC).
Results: Response in urinary and plasma MMA varied by smoking habit. For ever-smokers, urinary and plasma MMA decreased significantly in the 10ug group with no further improvement at higher doses, whereas for never-smokers a significant response was only observed in the 500ug group. Both plasma B12 and serum holoTC increased in response to 10µg B12 and showed a further increase with 500µg B12. Even after receiving 500µg B12 for 8 weeks, 8% and 12% of people had plasma B12 < 200pmol/L and serum holoTC < 37 pmol/L, respectively; 24% and 16% of people had plasma MMA >0.37pmol/L and uMMA > 2.0 µmol/mmol creatinine, respectively.

Discussion and conclusions: Whilst 10 µg of B12 can increase circulating B12 and holoTC in an elderly population with moderate B12 deficiency, higher doses are required to correct metabolic deficiency. The effects of smoking habit need further consideration.

WS1 06
Bioavailability of Vitamin B12 in fish roe; Characterization of a rainbow trout enzyme-resistant B12-binder

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Background: Though seafood is considered a rich source of vitamin B12, little is known concerning the bioavailability of this vitamin in seafood.
Aim: To explore the bioavailability of vitamin B12 in rainbow trout roe and to characterize any B12 binding proteins in the roe.
Method: We administered 1 pmol 57Co-labelled B12 (57Co-B12) - either free or completely bound to human recombinant haptocorrin (rhHC) or rainbow trout B12 binding protein - orally to rats, and 57Co-B12 in the feces was measured. Homogenized trout roe was incubated with 57Co-B12 and characterized with regard to B12 binding capacity, affinity and glycosylation. The protein was purified from roe fluid by affinity chromatography on B12-coupled sepharose. Protein stability at low pH and against treatment with increasing conc. of chymotrypsin and trypsin was investigated.
Results: Absorption studies in rats showed that 63%, 27% and 26% of the orally administered 57Co-B12 was excreted in feces when bound to trout B12-binder, human rhHC or unbound, respectively. This indicates a poor bioavailability for B12 bound to trout binder. Characterization of the B12-binder from trout roe suggests a glycoprotein with high resemblance to human HC and IF. In accord with the known properties of HC, the protein is stable at pH 2 and has binding affinity for cobinamide, but like IF the protein resists degradation by chymotrypsin or trypsin.
Conclusion: We observed poor bioavailability of vitamin B12 present in rainbow trout roe fluid, most likely caused by the presence of an enzyme-resistant B12 binding protein. Our results warrant further studies on bioavailability of vitamin B12 in seafood.

B-vitamins and choline in neuropsychiatric disorders

WS2 01
Evidence of abnormal folate metabolism, RFC1 polymorphism, and DNA hypomethylation in mothers of children with autism

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Objectives: To measure plasma transmethylation metabolites and DNA methylation status in a local cohort of autism and control mothers and to determine the frequency of folate-relevant polymorphisms in DNA from 530 case-parent triads and 560 controls obtained from the NIMH repository.
Methods: Fasting plasma samples from 58 local autism mothers and 80 matched control mothers were analyzed for folate, methionine, S-adenosylmethionine, S-adenosylhomocysteine, adenosine, and homocysteine by HPLC- EC. Genome-wide DNA methylation (as % 5-methylcytosine) was measured by LC/mass spectrometry. Candidate genes included MTHFR C677T, MTHFR A1298C, MTRR A66G, TCII C776G, and RFC1 A80G.
Results: Maternal DNA from the autism mothers was significantly hypomethylated and plasma homocysteine, adenosine, and S-adenosylhomocysteine were significantly elevated among autism mothers. In the case-control analysis of over 2100 repository DNA samples, the RFC1 G allele frequency was increased among case mothers, but not among fathers or affected children. Log linear analysis of the RFC1 A80G genotype within family trios revealed that the maternal G allele was associated with a significant increase in risk of autism whereas the inherited genotype of the child was not. Plasma folate levels were significantly reduced among the local autism mothers.
Conclusions: Taken together, these results support a broader paradigm of autism gene-environment interaction that encompasses the mother as a genetic/epigenetic case as well as a fetal environmental factor. Inclusion of maternal genetic/epigenetics in the autism gene-environment paradigm could provide new insights into the etiology of this complex disorder.

WS2 02– WITHDRAWN

P3 10
Vitamin B12 And Folate Intake, Homocysteine Levels And Their Association With Cognitive Functioning In Dutch Elderly People

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Background: Elevated plasma homocysteine (Hcy) levels have been reported as a possible risk factor for cognitive decline in community-dwelling elderly people. Vitamin B12 and folate play an important role in Hcy metabolism. Our B-PROOF[1]study provides the unique opportunity to study these associations in a large study population in which cognitive functioning was assessed through a battery of tests.
Aim: To evaluate the cross-sectional association of vitamin B12 and folate intake and Hcy levels with cognitive function in Dutch elderly (N = 2855) who participate in the B-PROOF study.
Methods: Our subjects had a mean age of 74.1 yr (SD 6.5), 50 % female, and median of 14.4 µ mol/L (IQR 13.0-16.6). Vitamin B12 and folate intake was measured with a validated Food Frequency Questionnaire. Cognitive functioning was assessed with the Mini-Mental State Examination (MMSE) and six specific cognitive tests. The results of these tests were combined into four cognitive domains; Attention and Working Memory, Information Processing Speed, Executive Function and Episodic Memory, using compound Z-scores. Multiple Linear Regression analysis was performed to examine the association of vitamin B12 and folate intake and plasma Hcy levels with cognitive domains (n = 856). The association of Hcy levels with global cognitive

functioning (MMSE) was calculated with Poisson-regression analysis (n = 2855).
Results: Hcy levels are inversely associated with cognitive functioning in the domains 'Information Processing Speed' (β = –0.018, p = 0.01) and 'Episodic Memory' (β = –0.09, p = 0.02). This would imply that if the Hcy level would be 10 μ mol/l higher, the participants would translate 2 symbols less in 90 seconds, need 3 more seconds to connect 25 numbers and recall 0.2 words less out of 15 words. Participants in the highest Hcy quartile (>16.5 μ mol/l) score 11 % lower on the MMSE test (Rate Ratio 1.11, p = 0.04) than participants in the lowest quartile (12–13 μ mol/l). Associations were adjusted for sex, age, education, smoking, alcohol intake and depression. We did not observe an association between folate or vitamin B12 intake and cognitive function (p > 0.05).
Discussion and Conclusions: We observed a modest inverse association of plasma Hcy levels with cognitive function in a Dutch population of elderly people. Vitamin B12 and folate intake were not associated with cognitive function.

[1] B-Proof is an acronym for B-vitamins for the prevention of osteoporotic fractures

WS2 03
Folic acid efficacy as an alternative drug added to sodium valproate in the treatment of acute phase of mania in bipolar disorder: a double-blind randomized controlled trial

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Objective: The purpose of this study was to evaluate the efficacy of using folic acid as an adjuvant therapy with sodium valproate in the manic phase of patients with bipolar disorder, in comparison with a control group treated with sodium valproate and a placebo.
Material and Methods: Following a double-blind randomized controlled trial, 88 clinically manic patients with diagnosis of type I bipolar disorder (BID) were divided randomly into two groups (case and control). The case group was treated with folic acid and sodium valproate and the control group with sodium valproate and placebo. The severity of mania was assessed using the Young Mania Rating Scale (YMRS) at the beginning and end of the first, second and third weeks of the study.
Results: Eighty-eight patients with BID (mean age 35.0 ± 8.4 years, M/F 1.4) were randomly divided into two groups. The two groups were similar regarding their age (P = 0.31), gender (P = 0.45), mean dosage of sodium valproate (1180 ± 240 mg vs. 1300 ± 300 mg, P = 0.68) and the initial YMRS scores (P = 0.74). From 44 patients in each group, 41 (93.2%) patients in the case group and 43 (97.7%) patients in the control group finished the protocol successfully. The case group's mean manic YMRS measurements (SD) before the initiation of therapy and in the first, second and third weeks of treatment were 34.0 ± 7.7, 26.7 ± 2.1, 18.1 ± 2.1 and 7.1 ± 0.9 respectively. The control group's measurements were 34.7 ± 3.8, 27.3 ± 2.3, 20.7 ± 2.5 and 10.1 ± 1.1. There was a statistically significant difference in YMRS scaling results between the case and control groups after 3 weeks of treatment (7.1 ± 0.9 vs. 10.1 ± 1.1, P = 0.005).
Conclusion: Based on our findings, folic acid seems to be an effective adjuvant to sodium valproate in the treatment of the acute phase of mania in patients with bipolar disorder.
Keywords: Mania, Folic Acid, Sodium Valproate, Bipolar Disorder

WS2 04
Folate, vitamin B12, homocysteine and polymorphisms of genes participating in one-carbon metabolism in late onset Alzheimer's disease

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Background: Increasing evidence points to one-carbon metabolism impairments in individuals with Alzheimer's disease.
Aim: We screened 378 late onset Alzheimer's disease (LOAD) patients and 308 matched controls for the presence of the common *MTHFR* 677C>T, *MTRR* 66A>G, *MTR* 2756 A>G, and *TYMS* 28bp repeat polymorphisms, searching for association with disease risk and age at onset. Moreover, we searched for correlation between each of the studied polymorphisms and available data on plasma homocysteine (hcy), serum folate and vitamin B12 values.
Methods: Genotyping was performed by means of PCR-RFLP technique. Correlation between the studied polymorphisms and biochemical data was performed by means of analysis of variance.
Results: A significant increased frequency of the *MTHFR* 677T allele (0.48 vs. 0.42; p=0.019) and of the *MTRR* 66G allele (0.49 vs. 0.43; p=0.044) was observed in the LOAD group. Significantly increased mean plasma hcy levels (22.7±1.7 vs 14.5±1.7 μmol/L; p=0.037) and decreased serum folate values (5.7±0.5 vs. 7.8±0.8 ng/mL; p=0.005) were observed in LOAD subjects with respect to controls. Significant interactions were also observed between the *MTHFR* 677T allele and both serum folate and vitamin B12 levels (p<0.05), between the *MTRR* 66G allele and vitamin B12 levels (p<0.01), and between the *MTR* 2756G allele and plasma hcy values (p<0.05).
Discussion and Conclusion: Overall, present results support a contribution for one-carbon metabolism to LOAD pathogenesis.

WS2 05
High prevalence of functional vitamin deficiencies in a psychogeriatric ward

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Background: Although vitamin B12 and folate affect cognition, little is known about functional deficiencies of vitamin B12 and folate in psychogeriatric patients.

Aims: 1) determine vitamin B12 and folate status.2) examine the relationship between methylmalonate (MMA) and vitamin B12.3) determine the relationship between vitamin B12 levels and use of proton pump inhibitors (PPI). 4) compare vitamin status between various diagnostic categories.
Methods: 71 consecutive patients admitted to a psychogeriatric ward were assessed for vitamin B12 (RR 133-680 pmol/L), MMA (RR < 0.4 µmol/L) [for vitamin B12 levels ≤ 220 pmol/L], red cell (RC) folate (> 356 nmol/L), homocysteine (HCY) (RR < 15 µmol/L) and creatinine. Diagnoses were determined according to DSM IV criteria (Major Depressive Episode [MDE] 31, schizophrenia 15/schizoaffective 4, Bipolar Affective Disorder [BPAD] 12, other 9). Vitamins and PPIs taken at admission were recorded.
Results: Three of 70 (4%) and 26 (37%) of patients had vitamin B12 < 133 and 134-220 respectively. Six of 25 (24 %) of patients with lower normal vitamin B12 had impaired MMA. Vitamin B12 levels were similar for patients on PPI and not on PPI. Median RC folate was 1127 ± 490 and two patients were folate deficient. 33 (46%) had elevated HCY including all patients with renal failure. Vitamin B12, RC folate and HCY did not vary between diagnoses of MDE, schizophrenia/schizoaffective disorder and BPAD.
Conclusions: Functional vitamin B12 deficiency existed within the normal reference range. Vitamin B12 levels were not related to use of PPIs. Folate deficiency was rare and elevated HCY was common especially in renal failure.

WS2 06
Homocysteine Intrahippocampal Injection-increased MDA, SOA, Bax levels as well as impaired Memory Retention in the Rat.

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Background: Homocysteine (Hcy) is a toxic metabolite of Methionine and its increased level in plasma, called *Hyperhomocysteinemia*. Recently the roles of Homocysteine in some neurodegenerative diseases for example Alzheimer's disease and Parkinson disease were investigated in some studies. In this study we investigated the effect of Homocysteine (Hcy) on oxidative stress in rat's hippocampus
Methods: Hcy was injected (0.2 micromoles) intrahippocampal in rat's brain. Five days after Hcy injection two parameters of oxidative stress, Malondy Aldehydes (MDA) and Super Oxide Anion (SOA) were analyzed in homogenate rat's brain with spectrophotometer .On the other hand the effect of Hcy on the Memory was studied by Passive Avoidance Learning Apparatus. Also Immunohistochemical assays were done on hippocampus slices of rat's brain.
Results: Results indicated that Hcy induced lipid peroxidation and increased malondialdehyde (MDA) and superoxide anion (SOA) levels significantly ($p< 0.05$) in the homogenate rat's brain. In addition, Hcy impaired memory retention in the passive avoidance learning test significantly. Also histopathological study revealed that Hcy could express Bax (Apoptotic biomarker) in DG layers of hippocampus.
Conclusion: These results suggest that Hcy may induce lipid peroxidation in hippocampus as well as impaired memory retention. In addition Hcy may induce Apoptosis and cell death in rat hippocampus.

B-vitamins and choline in gestation & birth defects

WS3 01
Paternal folate status and placental and fetal brain DNA methylation in the offspring

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To investigate the effect of paternal folate status on placental and fetal brain DNA methylation, rats were divided into four groups: paternal folate-supplemented (PS), paternal folate-deficient (PD), maternal folate-supplemented (MS), and maternal folate-deficient (MD). Folic acid-supplemented groups were fed with 8 mg folic acid/kg diet, while no folic acid was added to folate-deficient groups. Four weeks after the experimental diet started, rats were mated (PS×MS, PS×MD, PD×MS and PD×MD) and placenta and fetal brain were isolated at day 20 of gestation. The placental folate content decreased in the PD×MS (8.5 ± 0.46 nmol/g of wet tissue) group, as compared to the PS×MS (10.4 ± 0.59 nmol/g) group, followed by PS×MD (8.4 ± 0.36 nmol/g) and PD×MD (6.8 ± 0.37 nmol/g) groups ($P < 0.0001$). The fetal brain folate content in the PD×MD (1.05 ± 0.06 nmol/g) group decreased the most, and PD×MS (1.19 ± 0.07 nmol/g) group has been significantly lower than other groups (PS×MD; 1.36 ± 0.10 nmol/g, PS×MS; 1.37 ± 0.10 nmol/g, $P < 0.05$). The placental global DNA methylation decreased in the PD×MS group, as compared to the PS×MS group. The placental global DNA methylation was decreased the most in the PD×MD ($P < 0.0001$), and there was no difference between PS×MD and PD×MS groups. The fetal brain global DNA methylation in the PD×MD group was decreased most, followed by PD×MS group and PS×MD group as compared to the PS×MS group ($P < 0.0001$). In conclusion, paternal folate deficiency led to decreased folate content and global DNA methylation in the placenta and fetal whole brain, as similar to maternal folate deficiency and/or parents folate deficient group.
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WS3 02
Rapid Repletion for Preventing Birth Defects in Folate Deficient Women

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Initiation of neural fold closure occurs during Carnegie stage 10 and is completed by stage 12 (i.e., ~days 22 to 28 after conception). Examples of incipient human embryonic neural dysraphism have been reported only from Carnegie stage 11, or later. Currently, it is generally believed that women are not aware of being pregnant until it is too late to intervene with folate. However, many are now informed through home hCG test kits that can reveal the implanted state at about 14 days post conception or earlier. Typically, this would allow about a 7 day window of opportunity. AIM: To examine whether rapid folate repletion, which has heretofore been considered unachievable, might be accomplished with 5-methyl-6S-tetrahydrofolate (5-MTHF).

METHODS: Various dose regimes of 5-MTHF were studied using non-pregnant female volunteers, age 19-45 yr, screened to have lower than normal plasma folate levels. After 2 or 3 days (depending on the regime), subjects were switched to daily supplementation with folate in the amount of the RDA for prenatal vitamins. Levels of both total folate and 5-MTHF in plasma (the source of embryonic folate) were measured over the course of the treatment. RESULTS: Starting from about 30 min after the first dose of 5-MTHF, plasma folate could be elevated in a manner such that over the first three days it stayed above a value likely to fully decrease the risk of neural tube defects. Moreover, by 3 days of treatment, subjects could be switched to a prenatal vitamin, and still be continuously maintained above the minimum necessary plasma level. CONCLUSION: Rapid repletion of folate within a few days is possible, and may represent an option for women who have detected pregnancy within the critical window of opportunity.

WS3 03
Umbilical Choline And Related Methylamines Betaine And Dimethylglycine In Relation To Birth Weight

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Question: Low birth weight (LBW) is associated with increased morbidity and mortality for the newborn and increased risk on chronic diseases in adulthood. Choline has an essential role in the integrity of cell membranes, methylation reactions and memory development. We examined whether umbilical/maternal choline and related methylamines betaine and dimethylglycine (DMG) concentrations were associated with LBW in Dutch women.
Methods: Blood was sampled from umbilical cords at delivery (n=1126). Maternal blood was sampled at 30-34 weeks of gestational age (n=366). We calculated birth weights standardized for gestational age (SBW) and defined LBW as SBW <2500 grams.
Results: Maternal concentrations of all analytes were lower compared to umbilical cord concentrations. Plasma betaine and DMG between mothers and newborns were strongly correlated. Higher umbilical cord choline and betaine were associated with lower birth weight (β= -60[-89;-31] and β= -65[-94;-36]). Odds ratio for LBW was 4.12 [1.15;14.78] and 5.68 [1.24;25.91] for the highest umbilical choline and betaine quartile respectively compared to the lowest quartiles.
Conclusion: We observed an increased risk of lower birth weight with increased umbilical choline and betaine in venous umbilical cord blood. These results might reflect a change in choline consumption or metabolism or a disturbed placental function.

WS3 04
The activity and expression of enzymes of methionine synthesis are decreased in liver from foetuses with neural tube defects

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Background: Neural tube defects (NTD) are common malformations. Their pathogenic mechanisms are under the influence of genetic and nutritional risk factors. Maternal folate deficit and genetic determinants of the one-carbon metabolism increase NTD risk. The re-methylation of homocysteine in methionine is catalysed by two enzymes, methionine synthase (MTR) and betaine-Homocysteine MethylTransferase (BHMT). Methionine synthase uses vitamine B12 as coenzyme and methyl-folate as second substrate. Methionine is the precursor of S-adenosylmethionine (SAM), the universal methyl donor in transmethylation reactions.
Objective: To study the expression and activity of enzymes involve in the remethylation of homocysteine in methionine, methylenetetrahydrofolate reductase (MTHFR), MTR and BHMT and the concentration of S-adenosylmethionine (SAM) and folate and vitamin B12 in human liver tissue from NTD foetuses, compared with foetuses of spontaneous abortion.
Results: Decreased activities of MTHFR and MTR and decreased expression of MTR were observed in NTD tissues. BHMT activity was positively correlated with foetus age and MTR with SAM. The decreased concentration of SAM despite unchanged tissue concentration of folate, vitamin B12 and holoTranscobalamin confirmed the predominant role of decreased MTR in the decreased concentration of SAM.
Conclusion: Our results showed a decrease of activity and expression of enzymes of methionine synthesis, which may be a predisposing condition to NTD risk produced by maternal folate deficit. They are also consistent with the increased NTD risk associated with MTHFR, MTR and MTRR polymorphisms in population studies. The underlying mechanisms should deserve further attention.

WS3 05
MTHFR gene specific methyaltion in recurrent pregnancy losses

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Background: The etiology/ pathogenesis of recurrent pregnancy losses (RPL) via MTHFR C677T is still not clearly understood. Although studies suggest that the mutation results in the decrease in the enzyme activity by 40-70%, thereby resulting in the increase in the homocysteine (Hyperhomocysteinemia). In addition to this, DNA methyaltion is also reported to silence the activity of the gene. Thus genetic and epigenetic mechanisms of MTHFR gene are expected to

play an important role in complex disorders like recurrent pregnancy loss.
Aim: The present study attempts to understand the role of MTHFR C677T gene polymorphism and its methylation status in the causation of RPL in North Indian population.
Methods: A Case-control study was performed in north Indian population. Case group comprised of sixty-one (61) women with three (3) or more consecutive unexplained recurrent pregnancy losses before 24 weeks of gestation. Control group comprised of eighty-five (85) women with two (2) or more successful and uncomplicated pregnancies. Both the groups were analyzed for MTHFR C677T polymorphism and for its methylation status by Methylation Specific PCR amplification using primers that hybridize to the CpG island in the promoter region of MTHFR.
Results: Of all, number of individuals carrying methylated allele in promoter region of MTHFR among cases were found to be significantly high (32.79%) as compared to only 9.41% among controls (P<0.0001). Moreover, methylation status of the individuals carrying CT and TT (MTHFR Mutation) among cases is found to be significantly higher as compared to controls (p<0.001).
Discussion and conclusion: The present study suggests that the methylation at the promoter region of MTHFR plays an important role in the causation of recurrent pregnancy loss. Further, a woman carrying methylated allele, if has CT or TT genotype, is found to be more prone to recurrent pregnancy loss.

WS3 06
Peri-Partum Thiamine Lack Compromises Gestation Outcome And The Onset Of Maternal Behavior

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Background: A growing body of work refers prohormonal activities to the molecule of thiamine: Its lack caused several disorders such us ovarian dysfunction,[1] disturbances of the fetoplacental insulin-like growth factor (IGF-I) regulation,[2] unsuccessful fetus implantation and stillbirths,[3,4] long-lasting impairment of brain weight and function in growing pups[5] and altered circadian rhythmicity. [6] Recent discovery of thiaminylated adenine nucleotides[7] suggests its interactions with second messenger systems including cAMP (cyclic adenosine monophosphate), calcium mobilization and kinases activation.[8,9] Thiamine deficiency provokes also changes in behavior and induced either mouse-killing responses[10] (muricide) or disruption of maternal behavior via alterations of dopaminergic systems (unpublished data).
Aim: This study attempts to determine whether thiamine deficiency affects gestation outcome and maternal behavior towards pups.
Methods: Our investigations concern pregnant and lactating rats subjected to alternative different toxic effects of thiamine deficiency. Experiments compare thiamine-deficient diets-consuming females during pre, peri or postnatal periods with either controls or their respective pair-fed controls. Dams were observed for gestation outcome (spontaneous abortion, fetal death, litter size and birth weight)[4] and for apparent disorders of the maternal behavior related to the pups at parturition and within 48H after.
Results: Among the 7 experimental groups studied, only pre- and perinatal thiamine-deficient dams exhibited respectively: Spontaneous abortion (33.36 % vs. 41.66 %) and subsequent still births (43.3% vs. 56.6%), reduced litter size (72.32% vs. 76.16 %) and lowered birth weight (7 % vs. 30.1%), followed by pups-killing responses (57.14% vs. 71.43 %). These anomalies were not observed in the dams of any other experimental group and exacerbated significantly from pre- to perinatal period ($P < 0.05$ for any effect of thiamine deficiency compared with control and its own pair-fed control).
Conclusion: Thiamine lack around parturition compromises gestation outcome and the onset of maternal behavior. It may disturb both maternal and fetoplacental hormonal mechanisms perinatally induced.
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B-vitamins and choline in thrombosis & vascular diseases

WS4 01
Plasma homocysteine and the risk of venous thromboembolism: insights from the FIELD study

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Background: The lipid-lowering effect of fenofibrate is accompanied by a rise in plasma homocysteine, a potential risk factor for venous thromboembolism (VTE). This study investigated the relationship between homocysteine and the risk of VTE in patients treated with fenofibrate. Methods and results. The relationship between homocysteine and deep-vein thrombosis or pulmonary embolism was investigated in 9522 participants of the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial. All subjects received fenofibrate during a 6-week active run-in phase before randomization. A Cox proportional-hazards model was used to assess the effect of homocysteine on risk of venous thromboembolic events. During active-drug run-in, homocysteine rose on average by 6.5 µmol/L, accompanied by a substantial rise in plasma creatinine (+12%). Fenofibrate-induced changes in homocysteine and creatinine were fully reversible in the placebo group but persisted in the treatment group until the end of therapy. During follow-up, 1.7% had at least one episode of deep-vein thrombosis or pulmonary embolism: 103 on fenofibrate and 68 on placebo (log-rank P=0.006). In multivariate analysis, every 5 µmol/L higher baseline homocysteine was associated with 18.3 % higher risk of VTE. Fenofibrate treatment was associated with 51.6% higher risk, but the change in homocysteine with fenofibrate was not significantly associated with VTE after adjustment for baseline homocysteine. Baseline homocysteine and fenofibrate treatment interacted significantly. Conclusions Hyperhomocysteinemia is prospectively associated with VTE. Fenofibrate may predispose individuals with a high pretreatment

homocysteine towards VTE. The fenofibrate-induced increase in homocysteine did not completely explain the higher risk associated with fenofibrate therapy.

WS4 02
Hyperhomocysteinemia is associated with microvascular rarefaction in men, not in women. A population-based study

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Background: Homocysteine (Hcy) is an independent predictor of cardiovascular risk. The pathophysiological mechanisms underlying this link are not fully elucidated. Whereas the association between Hcy and vascular dysfunction in conduit arteries is extensively studied, the potential role of the microcirculation is largely unknown.
Aim: To assess the relationship of Hcy levels and microvascular structure and function in a population-based study in healthy, young adults.
Methods: We cross-sectionally studied 260 participants (aged 42 years, 47 % men) of the Amsterdam Growth and Health Longitudinal Study (AGAHLS). Nailfold videocapillaroscopy (100x) was used to assess capillary density (number of perfused capillaries/mm^2) at baseline, during venous congestion and during reactive hyperaemia (after 4 minutes of arterial occlusion). The relationship between fasting plasma Hcy and microvascular outcomes was evaluated using regression analyses with a dichotomous variable comparing the highest tertile with the two lowest tertiles of homocysteine with adjustment for BMI and blood pressure.
Results: Stratified analyses were performed for gender. In men, we observed an inverse, non-lineair relationship between Hcy and capillary density at baseline as well as during reactive hyperaemia, showing lower microvascular counts in the highest two tertiles of Hcy (β of -7.3 (c.i. -13.6 to -1.1) and -9.1 (c.i. -17.5 to -0.8) capillaries/mm^2 respectively ($p<0.05$)). In women, no such association was apparent.
Conclusions: In men, but not in women, elevated Hcy levels are associated with microvascular rarefaction, i.e. a decrease in the number of perfused capillaries at rest and during reactive hyperaemia.

WS4 03
Folate and B12 deficiency and high blood lead increase risk of hyperhomocysteinemia in individuals with *MTHFR* C677T transition in a Pakistani population

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Background: Genetic, nutritional and environmental factors could be contributing to highly prevalent hyperhomocysteinemia (>15µmol/l) in Pakistani population.
Aim: To unravel the underlying mechanism, we investigated the relationships of 6 SNPs in genes of enzymes involved in homocysteine metabolism - methylenetetrahydrofolate reductase (*MTHFR*; C677T, A1298C), methionine synthase (*MS* A2756G) and cystathionine β synthase (*CBS*, T833C/844ins68, G919A) with plasma homocysteine in an urban population in Pakistan and also studied the role of folate and vitamin B12 status and blood lead (Pb) levels in influencing homocysteine levels.
Methods: In a cross-sectional study, 872 healthy adults were recruited from a population in Karachi. Fasting venous blood was obtained and assessed for plasma/serum homocysteine, folate, vitamin B12, pyridoxal phosphate and blood Pb. DNA was isolated and genotyping was performed by PCR-RFLP based assays.
Results: *MTHFR* C677T transition was found to increase the risk of hyperhomocysteinemia [OR(95% CI); 10.17(3.6-28.67) for *MTHFR* 677TT genotype vs. 677CC genotype]. Protective effect towards hyperhomocysteinemia was observed with heterozygous (wild/insertion) genotype of *CBS* 844ins68 compared to homozygous wild type [OR(95% CI); 0.58(0.34-0.99]. *MS* A2756G, *CBS* G919A and *MTHFR* A1298C had no significant effect on plasma homocysteine. Individuals with *MTHFR* 677CT or TT genotypes were at a greater risk of hyperhomocysteinemia in folate and vitamin B12 deficiencies and high blood Pb level ($p<0.05$).
Conclusion: *MTHFR* C677T transition, folate and vitamin B12 deficiencies, male gender and high blood Pb level contribute towards the development of hyperhomocysteinemia in Pakistani population.

WS4 04
The correction of hyperhomocysteinemia and hyposelenemia in elderly patients with a stable stenocardia

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Background: The previous researches of the authors showed a combination of moderated hyperhomo-cysteinemia (HHCY) and severe hyposelenemia together with manifestation of endothelium dysfunction in patients with myocardial infarction. We showed the correction of specified disturbances and efficacy of standard therapy supplemented by the complex of vitamins, containing folic acid (FA), and Se as Se-methionine on the endothelial function and carotid artery stiffness.
Aim: To develop the given direction of research we examined 62 elderly patients (28 men and 34 women) at the age of 67,3 [62,6; 71,6] with stable stenocardia (functional class II) and grade II arterial hypertension, FC I-II (NYHA).
Methods: Plasma folate (SimulTRAC-SNB), HCY (HPLC), Se (EAAS) and vascular functional parameters (endothelium-dependent vasodilatation, arterial stiffness) were measured. All investigations were performed before and after 3 months of complex therapy, including perindopril, aspirin, bisoprolol, atorvastatin, 500 mg FA and original drug «SELENOBEL», containing 0,4 g diacetofenonilselenid (0,1 g Se).
Results: The target correction of HHCY and hyposelenemia together with standard therapy resulted in change of plasma folate concentrations from 7,4 [4,5;11,6] to 10,5 [8,5;12,6] nmol/l in men and from 6,9 [4,8;9,3] to 13,4 [9,6;15,7] nmol/l in women; HCY – from 16,5 [11,1;18,5] to 15,8 [11,1;17,0] mkmol/l in men and from 16,8 [10,8;20,4] to 11,8 [8,5;15,3] mkmol/l in women; Se - from

56,4 [44,3;70,2] to 70,3 [65,2;82,8] mkg/l in men and from 64,2 [50,6;71,5] to 81,3 [72,2;91,5] mkg/l in women.
Conclusion: Improvement of the biochemical status correlated with positive dynamics of vessels functional properties (mainly in elderly women).

WS4 05
Blood levels of homocysteine, vitamin B12 and folic acid in patients with type II diabets mellitus: possible correlation with endothelial dysfunction

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Background: Increased blood homocysteine (Hcy) is a risk factor for cardiovascular disease (CVD), which is a major complication and the main cause of death in patients with type 2 diabetes mellitus (DM). Endothelial dysfunction (ED), an established mechanism of CVD, is in essence the reduced bioavailability of endothelium-derived nitric oxide (NO).
Aim: We aimed to evaluate the relation, in DM patients with and without atherosclerosis (ATS), between ED and the concentrations of Hcy, folate, and vitamin B12 in blood serum.
Methods: 80 healthy subjects and 118 patients with DM were included in this study: 40 patients in the DM group had established ATS and the rest of 78 had no evidence of ATS. We used ELISA to measure blood serum concentrations of Hcy, B12, folate and nitric oxide (NO), as well as blood plasma concentrations of endothelin-1 (ET1).
Results: Hcy was higher in DM patients with ATS compared to DM without ATS and this correlated well with ED markers (ET-1, NO and Hcy were positively correlated). Correlation between serum NO and serum Hcy was stronger in DM patients with ATS (r=0.40) vs. control (r=0.10). In DM patients there was a negative correlation between Hcy and either B12 (r=-0.37) or folate (r=-0.43), compared to the positive one in healthy subjects (r=0.10 for Hcy and B12 ; r=0.21 for Hcy and folic acid).
Disscussion and Conclusion: We discuss the multiple facets of the correlations observed, including a possible link with the insulin resistance in type 2 DM. The correlation between serum NO and Hcy observed in our DM group with ATS could be important for ATS evolution and coronary events in DM. An early manifestation of vascular disease progressing to ATS in type 2 DM patients is ED and this may be related to hyperhomocysteinaemia.

WS4 06
Vitamin B_{12} level in peripheral arterial disease

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Objective: Hyperhomocysteinemia is considered a risk factor for atherosclerosis. Methyltetrahydrofolate reductase (MTHFR) gene mutation and low level of plasma vitamin B_{12} and folic acid could take part in the etiology of peripheral arterial disease (PAD).
Methods: We examined whether plasma vitamin B_{12} and folic acid levels and MTHFR C677T polymorphism are associated with the risk of PAD. The study comprised 293 patients (107 females, 186 males, mean age of 66±SEM0.7 years) and 293 sex matched controls (mean age of 62±SEM0.8 years) subjects. MTHFR genotypes were assessed by real-time polymerase chain reaction assay. We also determined plasma hsCRP, creatinie, vitamin B_{12}, folic acid and total homocysteine for all patients and controls.
Results: We adjusted all calculations for confounding parameters where it was appropriate. Accordingly, as determined by logistic regression analysis, no significant odds ratios for different genotypes of these polymorphisms could be observed. There was a significant lower level of vitamin B_{12} in PAD patients, and this was observed in males but not in females. 43% and 25% of patient and control populations, respectively, were in the lowest quartile of vitamin B_{12} (12 in the lowest quartile at least 10 fold increases the risk of PAD and it was independent of plasma folic acid level.
Conclusion: Low level of plasma vitamin B_{12} is an important determinant of hyperhomocysteinemia in PAD patients. PAD was not associated with an increased prevalence of MTHFR C677T mutation in the population studied.

B-vitamins, choline and cancer

WS5 01
B-vitamin intakes and incidence of colorectal cancer: Results from the Women's Health Initiative Observational Study Cohort

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Question: We investigated the associations between intakes of one-carbon nutrients and CRC in the Women's Health Initiative Observational Study, stratified by folic-acid fortification era and alcohol intake.
Methods: 88,045 postmenopausal women were recruited across pre- (1993-95), peri- (1996-97), and post- (1998) fortification periods, and incident CRC cases were ascertained as of 2009. Dietary intakes were assesed via a food frequency questionnaire and dietary supplement inventory. Hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated by Cox proportional hazards models.
Results: High versus low dietary and total intakes of vitamin B_6 (HR=0.80, 95% CI 0.66-0.97 and HR=0.80, 95% CI 0.66-0.99, respectively) and total intakes of riboflavin (HR=0.81, 95% CI 0.66-0.99) were

associated with reduced risk for CRC overall and regionally spread disease. In current drinkers who consumed less than one drink (13g alcohol) per week, but not non-drinkers or current drinkers who consumed one drink/week or more, folate, B_6, B_{12}, and riboflavin intakes were inversely associated with CRC risk (p for differences in slopes
Conclusions: B-vitamin intakes from diet and supplements were associated with decreased risk for CRC in postmenopausal women. The associations were particularly strong for regional disease and among women who consumed less than 1 alcoholic drink per week.
Figure 1: Time sequence of the national program of folic-acid fortification, WHI-OS recruitment and Food Frequency Questionnaire (FFQ) databases used for measuring folic acid intake. (Abbreviations: WHI-OS, Women's Health Initiative-Observational Study; FA, folic acid; DFE, dietary folate equivalent).

WS5 02
Dietary Intake of Choline and Betaine in Relation to Incident Colorectal Cancer in the U.S. Multiethnic Cohort

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Background: Epidemiologic evidence is limited and inconsistent for the association between dietary choline or betaine and the risk of colorectal neoplasia, possibly due to different effects of choline-containing compounds and their food sources.
Aim: To investigate the association of dietary intake of choline compounds and betaine with the risk of colorectal cancer (CRC) in a multiethnic population with diverse diets.
Methods: Baseline diet was assessed through a 180-item food frequency questionnaire in a population-based sample of 215,251 men and women in Hawaii and Los Angeles who were of Caucasian, African, Japanese, Latino, and Native Hawaiian ancestry, and aged 45-75 in 1993-1996. Choline and betaine intakes were estimated using the USDA food composition database. A total of 1,988 male and 1,691 female incident CRC cases identified through 2007 were analyzed using proportional hazards regression, with adjustment for other risk factors.
Results: Comparing the highest to the lowest quintiles of the nutrients, CRC was associated with total choline [adjusted relative risk (RR) = 1.10, 95% confidence interval, 1.00-1.20] and betaine intake (RR = 1.25, 1.13-1.38) among men only. In particular, intake of free choline and glycerophosphocholine among the five choline compounds was associated with CRC. The association of choline and betaine with CRC was significantly modified by the consumption of alcohol and also of folate in both men and women. No ethnic heterogeneity was observed.
Discussion and Conclusion: These findings suggest that higher levels of certain choline-containing compounds and betaine may be associated with the risk of colorectal cancer, depending on the levels of alcohol and folate consumption.

WS5 03
Choline and betaine intake and risk of lethal prostate cancer: incidence and survival

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Question: Choline is concentrated in prostate cancer cells and may affect prostate cancer through its roles in cell membranes and one-carbon metabolism. Higher plasma choline was associated with increased prostate cancer risk. No study has examined dietary choline and prostate cancer.
Methods: We examined choline and betaine intake and risk of lethal prostate cancer (e.g. metastases or death due to prostate cancer) among 47,896 men. Diet was assessed six times during 22 years of follow-up. In a case-only survival analysis, we examined post-diagnostic intake of these nutrients and risk of lethal prostate cancer among 4,282 men diagnosed with non-metastatic prostate cancer.
Results: In the incidence analysis, we observed 695 events during 879,696 person-years. Men in the highest quintile of total choline had a 70% increased risk of lethal prostate cancer compared to the lowest quintile (hazard ratio (HR): 1.70; 95% confidence interval (CI): 1.18, 2.45; p-trend: 0.005). In the case-only survival analysis, we observed 271 events during 19,694 person-years. Men who consumed the most choline after diagnosis had a nearly 2-fold increased risk of lethal prostate cancer (HR Q5 v Q1: 1.97; 95% CI: 1.05, 3.70; p-trend: 0.08). Betaine was not associated with lethal prostate cancer.
Conclusion: Dietary choline may increase risk of developing a lethal form of prostate cancer, and post-diagnostic intake of choline may promote progression of localized prostate cancer to lethal disease.

WS5 04
Folate and vitamin B12, and related biomarkers of one-carbon metabolism in association with prostate cancer risk

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Background: Recent epidemiological studies suggested that high folate and vitamin B12 concentrations may be associated with increased prostate cancer risk. Other factors of the one-carbon metabolism have not previously been studied in population-based studies in relation to prostate cancer.
Methods: Within JANUS, a prospective cohort in Norway (n=317.000) with baseline serum samples, we conducted a nested case-control study among 3000 prostate cancer cases and 3000 controls, matched on age at serum sampling, county of residence, and blood storage time. Subjects were screened for several different biomarkers of the one-carbon metabolism. Prostate cancer risk was estimated according to serum concentrations, using conditional logistic regression. To adjust for degradation during sample storage, folate concentration was measured as *p*-aminobenzoylglutamate equivalents following oxidation and acid hydrolysis.
Results: Neither folate nor vitamin B12 concentrations were associated with prostate cancer risk in overall analyses, although high folate concentration was associated with increased risk among individuals ≥50 years at serum sampling (highest vs. lowest quintile odds ratio (OR)): 1.37, P_{trend}=0.04. Conversely, high glycine and sarcosine concentrations were associated with reduced risk (glycine: OR=0.82, P_{trend}=0.07; and sarcosine: OR=0.87, P_{trend}=0.04). No associations were observed for homocysteine, methionine, betaine, or dimethylglycine.
Conclusion: This large-scale population-based study does not suggest that high folate and vitamin B12 concentrations affect prostate cancer risk. However, high concentrations of the related amino acids glycine and sarcosine may decrease prostate cancer risk.

WS5 05
Cross-talk between genome and epigenome influencing molecular phenotype and grade of breast cancer

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To explore the cross-talk between genome and epigenome, we have explored the expression of six genes i.e. RASSF1, RARb1, CCND1, BRCA1, p21 and BNIP3 in breast cancer cell lines treated with increasing concentrations of homocysteine. Dose-dependent down-regulation of RASSF1 and BRCA1 with homocysteine was observed in MCF-7 and MDA-MB-231 cells respectively. Using breast cancer tissues, we demonstrated down-regulation of RASSF1, RARb, CCND1 and p21; moderate expression of BRCA1; and increased expression of BNIP3. Breast cancer cases exhibited hypermethylation of RASSF1 (71.1 ± 6.3% vs. 30.0 ± 8.8%) and BRCA1 (95.0 ± 5.0% vs. 62.0 ± 12.7%) promoters. RASSF1 and BRCA1 methylation showed positive association with tubule formation and mitotic index respectively indicating their association with disease severity. Hyperhomocysteinemia was associated with hypermethylation of RASSF1 in ER and PR negative tumors while BRCA1 showed hypermethylation in all molecular phenotypes. RASSF1 methylation showed positive association with RFC1 G80A and SHMT C1420T; and inverse association with MTHFR C677T and MTRR A66G variants. BRCA1 methylation showed inverse association with GCPII C1561T variant while showing positive association with RFC1 G80A, cSHMT C1420T, TYMS 5'-UTR 28bp tandem repeat, TYMS 3'-UTR ins6/del6, MTHFR C677T and MTR A2756G variants. To conclude, aberrations in one-carbon metabolism influence epigenetic profile of two crucial genes i.e., RASSF1 and BRCA1 thus directly influencing breast cancer progression.

WS5 06
Application of the Vitamin Folic Acid as a Targeting Agent for Radionuclide Therapy of Folate Receptor Positive Cancer

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Background: The vitamin folic acid emerged as a valuable tumor targeting agent because of its specific accumulation in folate receptor (FR)-positive cancer. Thus, a number of folic acid radioconjugates have been developed for nuclear imaging purposes. However, due to undesired accumulation of folate-based radioconjugates in radiosensitive kidneys a therapeutic application was not possible so far.
Aim: The aim of this study was to preclinically investigate a novel folate radioconjugate with an albumin binding entity for radiotherapeutic purposes.
Methods: The folate conjugate was radiolabeled with the beta-particle emitting radioisotope ^{177}Lu. Cell uptake of the radioconjugate was studied in FR-positive KB tumor cells. An ultrafiltration assay was used to investigate plasma binding properties. The ^{177}Lu-radioconjugate was investigated in KB tumor bearing nude mice with regard to its tissue distribution and therapeutic anticancer efficacy.
Results: The ^{177}Lu-radioconjugate proved FR-specific uptake in cultured KB cells and significant binding to serum proteins. The tissue distribution of the ^{177}Lu-folate resulted in a high tumor uptake whereas accumulation in the kidney was significantly lower than usually found with other radiofolates. Therapeutic studies (20 MBq/mouse) in mice showed significant inhibition of tumor growth and a prolonged survival (> 2-fold) of treated mice compared to untreated controls.
Discussion & Conclusions: Installation of an albumin binding entity improves the tissue distribution of folate-based radioconjugates significantly. This approach allowed for the first time the performance of folate-based radionuclide therapy. The excellent preclinical results hold promise for future application of FR-targeted radionuclide therapy in patients.

Vitamin deficiency, supplementation, and clinical outcome

WS6 01
Lower homocysteine levels are associated with better quality of life scores in older people

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Background: Although there is evidence of a strong link between B12/folate status and possibly high levels of plasma homocysteine (tHcy) and depression in older adults less is known of a relationship between B12, folate and quality of life [1]. The aim of this report is to examine the associations between elevated plasma tHcy and quality of life scores in older patients recovering from acute illness.
Methods: Two-hundred and thirty-six hospitalised acutely ill older patients, who were part of a randomised double-blind placebo-controlled trial, were assigned to receive daily mixed oral nutritional supplements containing B-group vitamins or a placebo for 6 weeks. Outcome measures included quality of life measured using SF-36 scale and plasma Hcy levels.
Results: The mean tHcy concentration fell by 22% among patients given the supplements compared with the placebo group (mean difference 4.1 µmol/L (95% C.I, 0.14 - 8.03), p =0.043. tHcy concentrations was divided into 4 quartiles and analysed against quality of life scores. tHcy concentrations in the first relative to the fourth quartile of the distribution were associated with better quality of life scores at the end of the supplement period (SF-36 total score r = 0.25, p = 0.01).
Conclusions: Lower plasma tHcy concentrations were associated with better quality of life scores in older patients recovering from acute illness.
Reference: [1] Folstein M, Liu T, Peter I, Buel J, Arsenault L, Scott T, Qiu WW. Homocysteine hypothesis of depression. Am J Psychiatry 2007; 164: 861-867

WS6 02 presented as poster presentation P3 11

WS6 03
Urinary betaine excretion predicts excess loss of glomerular filtration rate in patients with diabetes mellitus

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Question: We have recently shown that urinary excretion of betaine is increased in diabetes mellitus and may be a marker of tubular

dysfunction, which has been associated with progression of kidney dysfunction in previous studies among diabetic patients. We therefore assessed if baseline urinary betaine excretion is a predictor of future loss of estimated glomerular filtration rate (eGFR) in diabetic or non-diabetic patients.
Methods: We studied 2133 patients (81.3% men) without macroalbuminuria or moderate renal failure. The majority had stable angina pectoris, and 225 had established type 1 or 2 diabetes at baseline. All participated in the Western Norway B-vitamin Intervention Trial (WENBIT). eGFR was calculated at baseline, at follow-up visits after 1 month and 1 year, and at the end of the study (mean 3.2 years). Multilevel linear modelling was used to calculate yearly change in eGFR.
Results: In patients with diabetes, urinary betaine excretion at baseline was a strong predictor of future loss of eGFR, p<0.001. At the geometric mean (SD) of betaine excretion (21.5 (8.1, 58.1) mmol/mol creatinine), the estimated (95% CI) yearly loss of eGFR was 1.39 (1.88, 0.91) mL/min/1.73 m^2. For each standard deviation increase in betaine excretion, we observed an additional loss of 0.84 (1.33, 0.34) mL/min/1.73 m^2, p=0.001 per year of follow-up. The result was not attenuated by adjustment for potential confounders, such as urinary albumin/creatinine ratio, baseline eGFR, age or gender. Betaine excretion did not predict loss of eGFR in patients without diabetes.
Conclusion: Urinary excretion of betaine is an independent predictor of excess loss of eGFR in patients with type 1 or 2 diabetes and cardiovascular disease.

WS6 04
Homocysteine in Older Adults: Association with Different Aspects of Physical Functioning

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Question: Growing evidence suggests that higher homocysteine levels are associated with lower physical functioning in older persons. The current study aimed to examine homocysteine in relation to different aspects of physical functioning.
Methods: Data from the B-PROOF study (cross-sectional) and the Longitudinal Aging Study Amsterdam (LASA) (cross-sectional and 3-year follow-up) were used. B-PROOF is a randomized controlled trial; LASA is an ongoing cohort study. The current study was performed in persons aged ≥65 years (N=1221-2878 in B-PROOF and N=321-1267 in LASA, depending on outcome). Different aspects of physical functioning, including physical performance, muscle mass, and grip strength were regarded as outcomes. Gender and serum creatinine were investigated as effect modifiers.
Results: In both LASA and B-PROOF, higher homocysteine levels were associated with lower physical performance, only in older women. Moreover, higher homocysteine was associated with decreased grip strength (B-PROOF) or loss of grip strength (LASA) in older women. The association with muscle mass was only found in LASA; in persons with serum creatinine levels below the median (classified as normal creatinine), homocysteine in the highest quartile was associated with an increased odds to loose muscle mass as compared with the first quartile.
Conclusions: In both studies, higher homocysteine levels were associated with lower physical performance and reduced muscle strength in older women. Minor differences might be explained by differences in the study populations' homocysteine range.

WS6 05
Pyridoxine (vitamin B6) therapy for premenstrualsyndrome

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Objective: A comparison between Pyridoxine (vitamin B6) and a placebo for the treatment of premenstrual syndrome (PMS).
Material and Methods: A double blind randomized clinical trial was performed on 160 university students who were suffering from PMS (according to the retrospective diagnostic criteria which had been recorded during the last 3 menstrual cycles). Then the patients were randomly assigned into two groups, and finally 94 patients who had finished the study were statistically analyzed.
In the Pyridoxine group (46 patients) vitamin B6 was prescribed at a dose of 40 mg twice daily (total 80 mg), and in the placebo group (48 patients) a tablet similar to vitamin B6 tablets in size, smell, shape and taste was prescribed 1 tablet twice daily. In both groups the tablets were started from the first day of the fourth menstrual cycle and continued for the next two cycles, and during these two cycles the symptoms were recorded.
Results: The severity of PMS in the second cycle of the treatment (in both groups) showed a statistically significant decrease (p <0.05, Pair T test) and the comparison between the two groups showed that the severity of PMS in the Pyridoxine group decreased more than the placebo group, which was statistically significant (p < 0.05, Student T Test) and this was because of the reduction in the psychiatric rather than somatic symptoms of PMS.
Conclusion: Regarding the effect of Pyridoxine in reducing the severity of PMS, it can be suggested as a treatment for PMS, at least for the psychiatric symptoms.

WS6 06
Folic Acid Paradox: Retrospective Record Review of Psoriatics and related skin disease on 1-6 mg Folic acid, Vitamin B6 and Vitamin B12

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Background: A psoriasis susceptibility locus has been asociated with the nitric oxide synthase (NOS) gene 2. When it is expressed, it produces inducible NOS (iNOS), the expression of which can be inhibitied by folic acid. iNOS also modulates contact dermatitis. Endothelial nitric oxide synthase (eNOS), from the NOS3 gene, exists in monomeric and dimeric forms. Coronary artery disease subjects in a study by others received either 400 mcg or 5 mg folic acid. The 400 mcg dose enhanced pro-inflammatory monomeric eNOS. The 5 mg dose significiantly enhanced anti-inflammatory dimeric eNOS.
Aim: To report the effects of low and high doses of of folic acid plus 1 mg B12 and 100 mg B6 on psoriasis and psoriasiform contact dermatitis.
Methods: Retrospective case review of 4 patients Three patients had coronary artery disease (2/3 with elevated homocysteine). One of the 3 had psoriasiform contact dermatitis with nail pitting. The fourth patient age 40 had psoriasis onset at age 35 with elevated homocysteine.

Results: Two of the 4 psoriatics had their skin disease flare on 1-2 mg daily folic acid. All 4 showed reduction of their body surface rash area on 5-7 mg folic acid beginning at 4-10 weeks. Maximum percent reduction in body surface area rash were 22 for the 40 year old on 4 mg folic acid, 43 (5 mg folic acid), 51 (for a 109-112 kg man with psoriasiform contact dermatitis with atopic diathesis on 6 mg folic acid and 79 on 5-7 mg folic acid.
Discussion and Conclusion: There can be a parodoxical worsening effect of daily low dose folic acid (1-2 mg) and a beneficial effect of 4-6 mg on psoriasis and psoriasiform contact dermatitis.

P6 21
Serum versus Red Cell Folate - What to do in Clinical Practice?

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Background: Folate status can be assessed by serum or red cell folate analysis. While serum folate is easily measured on high throughput automated analysers red cell folate requires an extra haemolysis step, which makes this test more labour intensive and subject to error. It is not well established how these two tests compare and if one of them should be preferred.
Aim: This study sought to compare the ability of serum and red cell folate to assess folate status using homocysteine as a functional marker of intracellular folate availability. In addition we looked at the responses of both parameters to therapeutic folate supplementation and to the introduction of mandatory fortification of flour in Australia in 2009.
Methods: Data was collated from 648 patients who had had measurement of folate and homocysteine at a network of hospitals across northern Sydney, Australia. Serum vitamin B12 and creatinine were also measured and the estimated GFR (eGFR) calculated. All measurements were made on a Roche Modular analyser. Longitudinal analysis of a series of folate deficient patients commenced on supplementation was performed. In addition, all folate results from a 12 month period before and after the introduction of mandatory fortification were evaluated from a total of 12713 subjects.
Results: Serum and red cell folate both demonstrated a significant independent association with homocysteine on multivariate analysis. Among patients with normal eGFR and vitamin B12, there was no difference in correlation coefficient with homocysteine between serum folate (-0.35; 95% CI -0.45 to -0.24) and red cell folate (-0.34; 95% CI-0.45 to -0.21). Comparison of area under the ROC curve for predicting elevated homocysteine showed a significantly better performance of serum folate (AUC 0.67) over red cell folate (AUC 0.58), $p<0.01$. Therapeutic folate supplementation of deficient individuals consistently normalised both parameters into the high-normal range without showing a relevant difference between the two. The introduction of mandatory folate fortification in Australia increased mean serum (31.2 to 38.5nmol/L) but not red cell folate levels (2564nmol/L pre-fortification, 2537nmol/L post- fortification) and reduced plasma homocysteine (11.0 to 10.3µmol/L). The prevalence of abnormally low folate levels was seen to decrease with both parameters (decrease of 68% for serum and 45% for red cell folate). Lastly, red cell, but not serum, folate was seen to demonstrate an unexpected negative correlation with haemoglobin. This appears to be an artefactual increase in patients with low haematocrit.
Discussion and Conclusion: Our results show that serum and red cell folate are both useful to assess folate status in humans and to monitor the response to supplementation. However, serum folate may better identify folate deficient patients. Given that serum folate also has analytical advantages over red cell folate, it may be the preferable test for high throughput laboratories without compromising diagnostic performance.

B-vitamins and choline in child development

WS7 01
Folate Receptor antibodies (FRAbs) in neurodevelopmental disorders

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Background: FRAbs are associated with neural tube defect pregnancies and cerebral folate deficiency in developmental disorders including Rett syndrome and autism spectrum disorders.
Aim: To determine mechanisms by which FRAbs affect neurodevelopment *in utero* and during weaning in a rat model.
Methods: We determined the distribution of FRAbs administered IP to dams on GD14 and its effect on ^{3}HPGA uptake in the fetus. Similarly, the localization of Abs administered on PND10, 11 and 12 in weaning pups and its effect on folate uptake in the brain was determined. Additional pups were reared and underwent behavioral testing to determine functional deficits.
Results: FRAbs administered to pregnant dams showed most of the Ab localization to uterine and placental tissue with associated reduction in folate uptake in the fetus. FR Abs administered during weaning accumulated in the hippocampus, cerebral cortex, and midbrain (20% each) with highest accumulation in the cerebellum (40%). Consistent with this, folate uptake was significantly reduced in the cerebral cortex, cerebellum and midbrain.
Discussion and Conclusion: The results suggest that in addition to antibody mediated inflammation, blocking of folate transport to the fetus or to specific areas of the brain as the likely primary mechanism by which the antibody exerts its effect. These results offer an explanation as to how pharmacologic folate alleviates the symptoms associated with FRAb autoimmune disorders. This has major implications in the prevention and treatment of developmental disorders.

WS7 02
Neurobehavioural effects of neonatal choline supplementation in mecp2-308 mouse model of Rett syndrome

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Rett syndrome is a pervasive developmental disorder, primarily affecting girls, that causes a wide variety of debilitating symptoms and for which and no cure currently exists. Mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2, a transcriptional repressor and activator) have been found to be responsible for about 90% of classical RTT cases. Although several MeCP2-target genes have been proposed, mechanisms leading to the severe, progressive and specific neuronal dysfunctions when MeCP2 gene is mutated remain to be elucidated. We wanted to investigate long-term effects of postnatal choline supplementation (from birth till weaning) in the truncated MeCP2-308 mouse model of Rett syndrome.

We exposed from birth till weaning male hemizygous (hz) mutant mice to choline supplementation (through dietary dam exposure) and evaluate neurobehavioural effects (locomotr activity, emotional profile, brain Nerve Growth Factor and Brain Derived Growth Factor levels, SAM/SAH ratios).
Adult male mutant hz mice showed a reduction of locomotor activity compared to wild type (wt) littermates. Early choline treatment restored wt-like locomotor activity levels in hz mice. Decreased levels of cortical mRNA NGF were also found in hz mice. Choline supplementation enhanced NGF and BDNF expression in cortical and hippocampal regions.
In agreement with previous reports on MeCP2 null mice, postnatal choline supplementation attenuates some of the behavioural and neurobiological abnormalities of the Mecp2-308 phenotype.

WS7 03
Choline requirements, nutritional intake and plasma concentrations in extremely preterm infants: retrospective analysis and first biochemical data

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Question: While accepted as an essential nutrient, the role of choline in preterm infant nutrition is unclear.
Methods: Estimation of the adequate choline intake (AI) of preterm infants from dietary recommendations for term infants, children, and adults. Retrospective analysis of the choline intake in inborn infants with <1000g birth weight or <28 weeks gestational age in 2006 and 2007 (n=93). Mass spectrometric analysis of choline in blood plasma from hospitalized preterm infants (n=55) compared to age-matched cord blood at delivery (n=35).
Results: Estimated AI was 25mg/kg/d. Day by day variability of choline supply was high and reached a plateau at d11 (median: 21.7mg/kg/d; 25th percentile: 19.6; 75th percentile: 23.9). Whereas individual supply at d0-3 was <10mg/kg/d in 85%, such values were frequently found even from d11 onwards. Intakes ≥25mg/kg/d were only achieved in 2-18% of infants. Whereas at delivery preterm infants had median plasma choline of 38µmol/L (25th perc.:27; 75th perc. 54) during neonatal intensive care routine blood suctions only comprized 18 (13; 22)µmol/L. 7 out of 55 samples were below 10µmol/L.
Conclusions: Choline intake does not meet requirements in hospitalized preterm infants, with frequent critical shortage until postnatal d10 and insufficient supply in the majority of infants later on. This results in decreased choline concentrations, even below adult values. The deficient intake and plasma concentrations demonstrated in this study may contribute to the neuro-developmental impairment often observed in extremely preterm infants. Future studies need to investigate the effects of improved choline supply on neuro-cognitive development.

WS7 04
Maternal Homocysteine In Association With Small For Gestational Age Offspring: Systematic Review And Meta-Analysis

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Question: Growth retardation in utero leading to small for gestational age (SGA) newborns, is associated with increased neonatal morbidity and mortality and with life-long consequences such as poor cognitive function and cardiovascular diseases. Maternal total homocysteine concentrations (tHcy) have been linked to a wide range of adverse pregnancy outcomes and could possibly influence birth weight. We performed a systematic review and meta-analysis on the association of maternal tHcy and birth weight.
Methods: A literature search of English, German and French publications using Pubmed database (January 1966-July 2010) revealed 78 abstracts. Search terms were: homocysteine AND (birth weight OR small for gestational age OR intrauterine growth retardation). Studies were eligible if information on maternal tHcy, birth weight and the possible association between maternal tHcy and birth weight was available. Effect size estimates were converted to odds ratios as estimate of the relative risk for a woman to deliver SGA offspring when maternal tHcy exceeds the 90th percentile.
Results: The search yielded 19 studies for analysis, consisting of 21,326 individuals. Pooled analysis resulted in a crude OR of 1.25[1.09;1.44]. If this estimate is expressed as a linear effect, it corresponds to -31[-13;-51]g for 1sd increase in maternal tHcy. Adjustment for known confounders was not possible but a tendency to decreased strength of association was observed in studies after adjustment for strong determinants.
Conclusion: Higher maternal tHcy concentrations are associated with a small increased risk for SGA offspring. The small estimated birth weight difference might be of little clinical relevance for the individual newborn, however, it could be of greater importance on a population level.

WS7 05
Vitamin B12 deficiency induce alteration in DNA methylation and protein expression in rats: A proteo-epigenetic study

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Background: Vitamin B12 (B12) is an important micronutrient, deficiency of which is associated with several disorders. Micronutrient deficiency *in-utero* is proposed to predict risk of complex disorders as DNA methylome is particularly vulnerable to B12 and folate deficiencies, as these are primary sources of methyl groups.
Aim: The aim was to ascertain if maternal B12 deficiency affects epigenetic reprogramming and thus forms a basis of complex diseases in adults.
Methods: We used methylated DNA immunoprecipitation assay, followed by high throughput sequencing on Illumina's Solexa platform, to identify differentially methylated regions and 2D-differential in gel electrophoresis followed by mass spectrometry to identify differentially expressed proteins in pups born to B12 restricted and control mothers.
Results: We identified about 1400 DMRs (at 5% FDR) in the liver tissue of B12 deficient pups compared to controls. At the proteome level, several proteins involved in lipid, carbohydrate and amino acid

metabolism were differentially expressed between the two groups and the expression of many of these were restored to control levels after rehabilitation with B12. We also identified a transcription factor that affects these pathways and western blot analysis revealed that the transcription factor expression is altered in B12 deficient group.
Discussion and Conclusion: This study is first of its kind as correlation of the effects of maternal nutrition on genome methylation and the corresponding proteome profile of the offspring would help us understand the potential mechanisms by which nutritional deficiency during early developmental stages, leads to changes in methylome and manifest diseases in adults.

WS7 06
Infantile Thiamine Deficiency And Its Role In Language Impairment In Children

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Background: Thiamine plays a central role in cerebral metabolism and is essential for brain development in infants. In November 2003, twenty infants with encephalopathy were hospitalized in pediatric intensive care units in Israel, due to feeding with a non-dairy thiamine-deficient infant formula. Two of them died from cardiomyopathy and the rest remained with severe neurological sequela. It was estimated that 600-1000 infants had been fed with the thiamine deficient formula. These infants were considered as high-risk and their development was subsequently monitored. A previous study followed 20 children from this group aged 2-3 years and found significant language delay.
Aim: To assess the linguistic function of children aged 5-6 years who had been exposed to thiamine deficiency in infancy but had not displayed any neurological signs at the time.
Methods: 59 children aged 5-6 years, fed with a thiamine deficient formula for at least one month, were compared with 35 healthy children, fed with a different milk substitute, matched for age and residential area. All children underwent standardized tests for language assessment that are sensitive to lexical and syntactic impairments in this age group.
Results: All mean scores of the language assessments were significantly lower for the thiamine deficient group. The 3 tests assessing syntactic movement ($p < .0001$), as well as the lexical retrieval test ($p < .0001$), indicated syntactic and/or lexical specific language impairment for >90% in the thiamine deficient group as compared to 8% in the controls.
Discussion & Conclusions: The results indicate that thiamine deficiency in infancy causes severe language disorders, and that dietary factors may be one of the causes for language impairment.

Molecular mechanisms

WS8 01
N-methyl D-aspartate receptors (NMDARs) in human red blood cell membrane as targets for homocysteine and homocysteic acid

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Background: Recently expression of NMDARs was reported in bone marrow and in circulating leucocytes, platelets and in rodent red blood cells (RBCs). Activation of these receptors by glutamate, homocysteine (HC) or homocysteic acid (HCA) results in Ca^{2+} uptake.
Aim: We have explored expression of NMDAR subunits during erythroid precursor differentiation and abundance and function of these receptors in circulating RBCs of healthy humans and patients with sickle cell disease (SCD).
Methods: We have used ex vivo erythropoietic maturation of mononuclear cells, and circulating RBCs from peripheral blood of healthy humans and SCD patients. Receptor subunits' expression was measured using qPCR, immunoblotting and flow cytometry. NMDAR function was assessed using live imaging, patch clamping and associated changes in cell volume, density and activation of Ca^{2+}-sensitive K^+ channels.
Results: Functional NMDARs are expressed in erythroid precursor cells and present in circulating RBCs. Receptor abundance and activity is significantly enhanced in SCD-RBCs compared to cells of healthy subjects. Activation of NMDARs by treatment with agonists in vitro or during hemolytic crises in vivo triggers Ca^{2+} uptake, dehydration and "sickling" of SCD-RBCs. Treatment of SCD-RBCs with antagonists of NMDARs reversed these effects and reduced Ca^{2+} uptake and HbS polymerisation.
Discussion and Conclusion: Our data raise significant concern regarding the consequences of up-regulated plasma HC and HCA levels in SCD patients. Hyperactivation of NMDARs in circulating SCD-RBCs causes dehydration, increase in aggregability of RBCs, activation of Ca^{2+}-sensitive proteases contributing to vaso-occlusive crises in this group of patients.

WS8 02
Analysis Of The Enzymatic Activity Of Mutant Cbs Proteins Reveals A Strong Association With The Localization Of The Affected Residue In The Protein Primary Structure

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Background/Aim: The key regulatory point of transsulfuration is catalyzed by cystathionine beta-synthase (CBS), a homotetrameric PLP-dependent enzyme activated by S-adenosyl methionine (SAM). Each CBS monomer comprises three domains, an N-terminal domain, a catalytic core and a C-terminal regulatory domain. Previously we characterized the genotypic background of a group of CBS deficient patients and developed a prokaryotic expression system to study the identified mutant proteins. Here we present the enzymatic activity, SAM activation ratio and oligomeric profile in crude *E. coli* extracts of this group of mutant CBS proteins.
Methods: CBS cDNA was cloned into pET28b expression vector. The mutations P49L, G151R, G153R, K269del, I278T, R336C, R336H, G351R, P227L, D444N, S500L and L540Q were introduced by site-directed mutagenesis. Enzyme activity was determined in crude *E. coli* extracts in the absence and presence of SAM (SAM activation ratio). Oligomeric profile was determined by Western blot analysis of native-gels.
Results/Discussion: We found a strong association between the enzymatic activity of the mutant protein and location of the affected residue. Mutations in the catalytic domain lead to inactive or lowly active proteins, whereas C-terminal domain mutations display a near normal activity and mutations within the N-terminal domain present an intermediate activity. Interestingly, no link between SAM stimulation and the location of the mutation was found. Further comparative analysis of the recombinant expression results with homozygous

patients' fibroblast data showed an identical behaviour, therefore validating our system.

WS8 03
Cystathionine gamma-lyase-deficient mice as an animal model of cystathioninuria/-emia; the comparison with cystathionine beta-synthase-deficient mice

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Cysteine is considered as one of the non-essential amino acids in mammals because it is synthesized from methionine via methionine cycle/transsulfuration. Cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE) are the transsulfuration enzymes essential for homocysteine metabolism/cysteine biosynthesis, and are currently attracting much attention as the major H_2S-producing enzymes. Although CBS-deficient mice had been generated as an animal model of homocystinuria, most of them die within 4 weeks after birth probably due to severe hepatic dysfunction, hampering the detailed analyses of these mice. We succeeded to obtain more viable CBS-deficient mice by altering its genetic background to C3H/HeJ; the mice appeared normal in general behavioral tests but showed cerebellar malformation and impaired learning ability. We also generated CSE-deficient mice as animal model of cystathioninuria/-emia; they appeared normal but displayed hypercystathioninemia/hyperhomocysteinemia though not hypermethioninemia. They displayed vulnerability to oxidative injury, acute lethal myopathy in cases of dietary cysteine deficiency, or acute lethal hepatitis in cases of dietary methionine excess. Because current newborn screening for homocystinuria detects methioninemia but not homocysteinemia via either simple Guthrie's method or tandem mass spectrometry, CSE-deficient patients may pass the screening but may suffer homocysteinemia and display severe pathological conditions upon amino acid-imbalanced diets.
References: Akahoshi et al., Hum Mol Genet 17:1994-2005 (2008); Ishii et al., J Biol Chem 285:26358-26368 (2010).

WS8 04
Cystathionine-β synthase deficiency increases collagen *N*-Homocysteinylation in mice

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Background: Clinical manifestations of severe hyperhomocysteinemia due to cystathionine-β synthase (*CBS*) deficiency include connective tissue abnormalities affecting skin, bone, lung, eye, and vasculature (Mudd et al. *Am J Hum Genet* 1985;37:1). Similar abnormalities are observed in *Cbs*$^{-/-}$ mice (Hamelet et al. *Exp Mol Pathol* 2007;83:249), but molecular mechanisms underlying these abnormalities remain obscure.
Aim: We predict that *N*-homocysteinylation of collagen lysine residues will prevent the formation of essential cross-links and cause connective tissue abnormalities. Because connective tissues are supramolecular assemblies, even low levels of *N*-Hcy-lysine in collagen can result in a structural defect.
Methods: We studied *Tg-I278T Cbs*$^{-/-}$ mice (tHcy=272±50 μM), which have elevated *N*-Hcy-protein (16.6±4.1 μM), and *Tg-I278T Cbs*$^{+/+}$ mice (tHcy=1.9±1.6 μM), which have normal and *N*-Hcy-protein (1.6±0.3 μM) (Jakubowski et al. *Faseb J* 2009;23:1721). We prepared collagen from skin of these mice using the acetic acid extraction method, confirmed its purity by SDS-PAGE, and analyzed its *N*-Hcy-collagen content.
Results: We found that *N*-Hcy-collagen was elevated 18-fold in *Tg-I278T Cbs*$^{-/-}$ mice, compared with *Tg-I278T Cbs*$^{+/+}$animals (89.9±25.1 *vs.* 5.0±2.4 pmol/mg skin).
Discussion and Conclusion: These findings demonstrate that collagen is a target for *N*-homocysteinylation *in vivo* in mice and can account for connective tissues deficiencies observed in severe hyperhomocysteinemia.

WS8 05
A novel function for an old folate enzyme

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Glycine N-methyltransferase (GNMT), an abundant cytosolic enzyme and a major folate binding protein, transfers the methyl group from S-adenosylmethionine (SAM) to glycine producing S-adenosylhomocysteine and sarcosine. It functions as a metabolic regulator of the availability of activated methyl groups. Importantly, mouse knockout studies and epidemiological data pointed toward GNMT as a cancer susceptibility gene.
We studied the role of GNMT in regulation of cellular proliferation. We expressed the protein and its mutants, deficient in catalysis, folate binding or cytoplasmic targeting, in several GNMT-deficient cell lines and evaluated proliferation, apoptosis, and distribution of cells between cell cycle phases. Using fluorescence-based techniques we also monitored intracellular compartmentalization of the enzyme.
This study demonstrated that GNMT is strongly down regulated in various human cancers while the re-expression of GNMT in cancer cell lines inhibits proliferation and induces apoptosis. Antiproliferative effects of either the catalytically deficient or folate binding deficient mutants of GNMT were the same as the wild-type enzyme. The growth-suppressor effects of GNMT were not rescued by high folate or methionine supplementation. We also showed that the enzyme is localized in both cytoplasm and nuclei. Furthermore, GNMT engineered to localize to nuclei only still evokes antiproliferative effects. Our findings indicate that the suppressor effects of GNMT were not caused by the depletion of SAM or restriction of availability of intracellular folates. We suggest that GNMT localized in the nucleus is responsible for the proliferation regulatory effects and that these effects require interaction of GNMT with specific nuclear proteins.

WS8 06
Formate and one-carbon metabolism

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Background: Impairments of the one-carbon metabolism (1C), due to vitamin B-deficiencies or defects in genes that encode enzymes, are associated with numerous pathologies and developmental anomalies. We have recently discovered that plasma formate concentration is elevated during vitamin B12 and folate deficiencies (1).
Aim: Our objective is to establish a robust assay for formate and to use this assay to determine its response to B-vitamin deficiencies, as well as to determine rates of whole-body formate metabolism in rats.

Methods: Vitamin B deficiencies were generated in rats by feeding deficient diets. Plasma formate was measured by GC-MS and homocysteine by HPLC. The whole-body kinetics of formate were determined by means of a constant infusion of 13C-formate.
Results: Formate determination by GC-MS was superior to that by formate dehydrogenase, particularly at low formate levels. Plasma formate was markedly elevated during folate- and vitamin B12-deficiencies but was unaffected by vitamin B6-deficiency. The entry of new formate into the formate pool is estimated to be approximately ~10 µmol/hr in ~200 g chow fed rats.
Discussion & Conclusion: Plasma formate concentration is very sensitive to disturbances in remethylation but not in transmethylation. Thus, it conveys information complementary to that obtained from Hcy and methylmalonic acid. Determination of formate kinetics in vitamin-B12- and folate-deficient animals will permit us to establish whether a defect in formate production or removal is responsible for the hyperformatemia of these conditions.
Reference: [1] Lamarre SG, AM. Molloy, SN Reinke, BD Sykes, ME Brosnan and JT Brosnan (in press) Formate can differentiate between hyperhomocysteinemia due to impaired remethylation and impaired transsulfuration. American Journal of Physiology; Endocrinology and Metabolism. doi:10.1152/ajpendo.00345.2011

Clinical outcome & metabolom after vitamin treatment

WS9 01
Reduced remethylation in patients with chronic kidney disease (CKD) induces vitamin B6 deficiency by enhanced trans-sulfuration activity

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Background: Hyperhomocysteinemia and vitamin B6 deficiency reduce bone quality in patients with chronic kidney disease (CKD).
Aim: To assess the link between hyperhomo-cysteinemia and vitamin B6 deficiency in CKD.
Methods: Ten hemodialysis (HD) patients were randomized to receive methylcobalamin 500 µg iv after HD and oral folic acid (FA) 15 mg/day po (Group A, n=5) or methylcobalamin and FA plus vitamin B6 (B6) 60 mg/day po (Group B, n=5) for 3 weeks. A methionine (Met) loading test was conducted before and after treatment and plasma levels of amino acids, such as homocysteine (Hcy), Met and cysteine (Cys), were measured. Serum levels of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) were also measured in 20 HD patients receiving the Group B regimen.
Results: B6 levels were significantly lower in HD patients than controls (2.9±1.1 ng/mL vs 20.1±10.8 ng/mL, $p<0.01$). Hcy levels were higher when fasting (30.3±10 nmol/mL vs 12.3±5.8 nmol/mL in controls, $p<0.01$) and demonstrated a greater increase on Met loading. Both treatments normalized the Hcy profile. The Cys level was also significantly elevated. The Met/Hcy ratio (representing remethylation activity) was lower in HD patients, but was increased by both treatments. The Cys/Hcy ratio (represents trans-sulfuration activity) was unaffected in HD patients, and was also increased by both treatments. Serum SAM (441.1±101.8 nmol/L) and SAH (340.1±91.1 nmol/L) levels were very high and increased after vitamin supplementation.
Conclusion: In CKD, the trans-sulfuration pathway was not attenuated, but was accelerated by Hcy-lowering therapy. Reduced remethylation induces B6 deficiency by enhancing trans-sulfuration, possibly due to an accumulation of SAM.

WS9 02
Kinetic Modelling of Storage Effects on Biomarkers Related to B-vitamin Status and One-Carbon Metabolism

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Background: Biomarkers and metabolites related to B-vitamin function and one-carbon metabolism have been studied as predictors of chronic diseases in studies based on samples stored in biobanks. For most biomarkers, stability data are lacking or fragmentary.
Methods: Degradation and accumulation kinetics of 32 biomarkers were determined at 23°C in serum and plasma (EDTA, heparin and citrate) collected from 16 individuals and stored for up to 8 days. In frozen serum (-25°C), stability was studied cross-sectionally in 650 archival samples stored for up to 29 years. Concentration versus time curves were fitted to mono-exponential, bi-exponential, linear, and non-linear models.
Results: For many biomarkers, stability was highest in EDTA plasma. Storage effects were similar at room temperature and at -25°C; notable exceptions were methionine, which could be recovered as methionine sulfoxide, and cystathionine, which decreased in frozen samples. Cobalamin, betaine, dimethylglycine, sarcosine, total homocysteine, total cysteine, asymetric and symmetric dimethyl arginine, creatinine and methylmalonic acid were essentially stable under all conditions. Most B-vitamins (folate, vitamin B_2 and B_6) were unstable; choline increased markedly, and some amino acids also increased, particularly in serum. The kynurenines showed variable stability. For many biomarkers, degradation (folate and flavin mononucleotide) or accumulation (pyridoxal, riboflavin, choline, amino acids) kinetics at room temperature were non-first order.
Conclusion: Data on stability and deterioration kinetics for individual biomarkers are required to optimize procedures for handling serum and plasma, and for addressing preanalytical bias in epidemiological and clinical studies.

WS9 03
Effect of One Year D- and B-vitamins Supplementation on Bone Metabolism

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Introduction: Vitamins D andB deficiencies and hyperhomocysteinemia are risk factors for osteoporosis. The benefit of B-vitamin

supplementation onbone metabolism is not well studied and is thought to be dependent on vitamin D status. This study investigates the effect of 1 year combined oral supplementation of the vitamins B and D on bone markers.
Methods: 111 subjects (mean age 69 years) were recruited from a rehabilitation program. The study was randomized and double blind. The treatment arms were: group A recieved 50 mg B_6, 500 µg B_{12}, 456 mg calcium, and 1200 IE vitamin D; group B received 1200 IE vitamin D and 456 mg calcium. Homocysteine (Hcy), metabolites, vitamins,parathormon (PTH), lipids, and bone markers [osteocalcin, bone alkaline phosphatase (BAP), tartratresistent acid phosphatase (TRAP), desoxypyridoniline (DPD), sclerostin] were determined in serum, plasma, or urine.
Results: At baseline, high Hcy, low vitamin D3 but elevated plasma PTH had been observed. A marked increase of 25-hydroxy vitamin D3 was observed in both groups in group A (16 to 32 ng/ml), in group B (16 to 29 ng/ml). As a consequence, high baseline PTH dropped to normal. Baseline Hcy was elevated (> 12 µmol/L) in 50% of the people and was normalized only in the group A receiving the B-vitamins. The bone metabolism was lowered after the treatment, markers of bone formation (BAP, osteocalcin) as well degradation markers (DPD, TRAP) decreased. Furthermore, the decline of osteocalcin in-group A was somewhat less than in group B. The changes in bone markers were not remarkable after 6 months of vitamin treatment.
Conclusion: Supplementation with vitamin B and D in elderly subjects had a beneficial effect in lowering risk factors of osteoporosis like Hcy and PTH, which affect bone degradation. There was a tendency that the bone turnover by combination of D and B-vitamins was not as high as by using of vitamin D alone. Because of the slow bone metabolism, longer duration of D and B-vitamin treatment should be tested.

WS9 04
Folic acid supplementation may disturb the regulatory effect of S-adenosylmethionine on methylenetetrahydrofolatereductase activity.

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Background: Elevated plasma levels of homocysteine (Hcy) are associated with an increased risk of cardiovascular disease. Supplementation with folic acid lowered plasma homocysteine, but failed to reduce the risk on cardiovascular disease.
Aim: Determine the effect of folic acid supplementation on the intracellular levels of one-carbon (1C) metabolites.
Method: Fifty healthy volunteers received either 500 µg folic acid daily for 8 weeks, or a placebo. Plasma and peripheral blood mononuclear cells (PBMC) levels of S-adenosylmethionine (SAM), S-adenosyl-homocysteine, Hcy, methionine, cystathionine and 5-methyltetrahydrofolate levels were measured by LC-MS/MS.
Results: At baseline, plasma levels are a poor reflection of intracellular levels of most 1C metabolites. Only cystathionine (R=0.445, P=0.001), 5-methyltetrahydrofolate (R=0.329, P=0.024) and Hcy (R=0.350, P=0.014) showed a significant correlation between plasma and intracellular levels. Folic acid supplementation did not significantly alter the PBMC levels of any of the 1C metabolites. Multiple regression analysis showed that baseline PBMC Hcy levels are correlated to PBMC SAM, PBMC methionine, PBMC 5-methyltetrahydrofolate and plasma Hcy levels. After folic acid supplementation, PBMC Hcy was correlated to PBMC SAH and plasma Hcy, suggesting a loss of SAM's regulatory function. In-vitro experiments confirmed that at higher folate substrate levels, physiological levels of SAM no longer inhibit MTHFr.
Conclusion: The effect of administration of folic acid is much different intracellularly than in plasma. In fact, intracellular 1C metabolism may become perturbed after folic acid supplementation

WS9 05
Supplementation With Cyanocobalamin In Stable Angina: A Novel Pathway For Asymmetric Dimethylarginine Breakdown?

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Background: Asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid and a component of the blood. ADMA is an endogenous inhibitor of nitric oxide synthesis, and its plasma level (P-ADMA) is elevated in patients with stable angina (SA), being a marker and a mediator of endothelial dysfunction in those patients. ADMA is believed to be eliminated mostly via degradation by dimethylarginine dimethylaminohydrolases. We suggested that an alternative, namely, demethylation, pathway may exist for ADMA disruption, with cyanocobalamin (Cbl) being a methyl group acceptor.
Aim: To check if Cbl intake may lead to P-ADMA decrease and associated elevation of plasma methylcobalamin (P-MetCbl) in SA patients different from controls.
Methods: 24 SA patients (P-ADMA:) and 25 age- and gender-matched controls were given Cbl per os at 500 µg 2x a day for 2 weeks. P-ADMA and P-MetCbl were re-measured on the day 15 by HPLC.
Results: In the control subjects, the intake of Cbl has lowered P-ADMA from 0,51±0,07 µM/L to 0,40±0,05 µM/L (by 18%, p<0,05) and increased P-MetCbl from 295±68 ng/L to 580±89 ng/L (by 97%; p<0,05). In SA patients, P-ADMA decreased from 0,70±0,09 µM/L to 0,42±0,08 µM/L (by 40%; p<0,05) and P-MetCbl rose from 204±87 ng/L to 760±107 ng/L (by 273%; p<0,05).
Discussion and conclusion: Administration of Cbl results in a decline of P-ADMA in both control and SA groups (more pronounced in SA). This was associated with a substantial increase of P-MetCbl (more pronounced in SA), while the elevation of plasma level of 5'-deoxyadenosylcobalamine was less marked. The findings indirectly support the possibility of existence of a demethylation pathway for the degradation of ADMA that is more active in SA patients compared to controls.

WS9 06
Dietary folate, vitamin B_{12} and vitamin B_6 affect both plasma choline and plasma DHA concentrations in rats

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Background: Folate, vitamins B_{12} and B_6 are essential nutritional components to support transfer of one-carbon units and therefore could support methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) by PE-*N*-methyltransferase (PEMT) in

the liver. It has been suggested that PC synthesis by PEMT cannot only affect choline availability but also influence circulating levels of polyunsaturated fatty acids, like docosahexaenoic acid (DHA) (Watkins *et al.*, 2003).
Aim: To investigate whether dietary enrichment with the three B-vitamins could increase plasma choline and DHA levels in rats.
Methods: Exp1: Plasma free choline, total DHA and total homocysteine (tHcy) were measured in rats that consumed a B-vitamin-poor diet for 4 weeks after which they were either continued on the B-vitamin-poor diet or switched to a B-vitamin-enriched diet for another 4 weeks.
Exp2: Plasma total DHA and tHcy were measured in rats after feeding them one of 4 diets with increasing levels of B-vitamins for 4 weeks.
Results: The results demonstrate for the first time that rats supplemented with B-vitamins show higher plasma choline and DHA concentrations. Moreover, plasma DHA was found to be dose-dependently increased by dietary B-vitamins.
Conclusion: The current data demonstrate the interdependence between B-vitamin intake and plasma choline and DHA, possibly mediated by enhancing PC synthesis by PEMT. Details on this mechanism, clinical implications, and pending additional results will be presented.

Folate physiology and methods

WS10 01
Clinical Recognition and Aspects of the Cerebral Folate Deficiency Syndromes

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Cerebral Folate Deficiency Syndrome (CFDS) represents a group of neuro-psychiatric disorders associated with low spinal fluid ^{5}N-methyltetrahydrofolate (MTHF) in the presence of normal folate and B_{12} status outside the nervous system. The most compelling underlying cause of CFDS is the presence of serum autoantibodies of the blocking and binding type directed against and forming complexes with the folate receptor (FR) antigen attached to the plasma-side of choroid plexus epithelial cells, which normally mediates the transport of MTHF to the CNS. Alternative causes of CFDS are FOLR-1 mutations and mitochondrial disorders.
Two important clinically recognizable syndromes of FR-antibody mediated CFDS have been detected in young children, being the infantile-onset CFD syndrome presenting 4 to 6 months after birth and a spastic ataxic syndrome which may present from the age of 1 year. In addition, serum FR autoantibodies and CFDS have also been reported in a proportion of children affected by a variant of the Aicardi-Goutières syndrome, Asperger syndrome, PDD.NOS, Rett syndrome and childhood disintegrative disorder (Heller's dementia). Moreover, we have identified within the autism spectrum disorders, CFDS due to FR auto antibodies as an important neurobiological marker for infantile autism associated with or without neurological deficits.
The heterogeneity of the clinical phenotype associated with FR-autoantibody mediated CFDS might be explained by differences in the time of onset and period during which these FR autoantibodies are generated and lead to folate deficiency within the nervous system. Folate deficiency during various critical stages of fetal development and infancy could have profound effects on the structural and functional refinement of the brain. Public awareness of CFDS in association with FR autoimmunity should lead to early detection and diagnosis with improved prognosis of these potentially treatable new group of neuro-psychiatric disorders.

WS10 02
Folate receptor alpha is crucial for folate transport across the blood-CSF barrier

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Background: Folates are essential nutrients and necessary cofactors for important metabolic pathways such as synthesis of amino acids, DNA and lipophilic substances. The major biological active metabolite 5-methyltetrahydrofolate (MTHF) is distributed by the blood stream. Active MTHF transport into the brain occurs at the choroid plexus and results in a twofold higher MTHF concentration in cerebrospinal fluid (CSF) than in plasma.
Aim: Reduced MTHF concentrations in the human CSF are associated with various neurological symptoms such as epileptic seizures, developmental delay and movement disorders. We aimed to disclose the interrelation between the clinical picture associated with reduced MTHF concentration and genetic alterations in MTHF transporting proteins.
Methods: We studied a group of pediatric patients with progressive neurological symptoms as well as severely reduced folate concentrations in the CSF. We assessed brain myelination by MRI and metabolic state by in vivo MR spectroscopy. Patients with brain abnormalities were screened for mutation in theFOLR1gene coding for folate receptor alpha (FRalpha).
Results: In 11 patients we identified mutations in the FOLR1 gene. The identified mutations either resulted in loss of FRalpha expression or in intracellular mistargeting of mutant FRalpha. Folinic acid therapy could restore CSF MTHF concentrations, reverse white matter alterations and consecutively improve clinical symptoms.
Discussion and conclusions: Mutations in the FOLR1 gene coding for FRalpha are responsible for inherited cerebral folate transport deficiency, a treatable childhood neurodegenerative disorder.

WS10 03
Regulatory mechanisms of folate transport in a rat model of folate oversupplementation.

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Background: Folic acid is key one-carbon donor required for numerous biological function, the deficiency of which is associated with the risk of various diseases. Countries with obligatory folate fortification of food have documented a significant decrease in neural tube defects. Food fortification along with nutritional supplements creates a state of folate oversupplementation, the effect of which on the folate absorption and regulation has not been studied.
Aim: To examine the effects of folate oversupplementation on the intestinal and renal folate transport in rats.
Methods: Male Wistar rats in group I were given semisynthetic diets containing 2mg folic acid/kg diet (control) and those in group II were given folate oversupplemented rat diet (20mg folic acid/ kg diet) for 10 days (short-term treatment) and for 60 days (long-term treatment).
Results: Folate uptake in intestine and kidney was significantly reduced in short-term folate oversupplemented rats as compared to control rats which was associated with a significant decrease in the

expression of the reduced-folate carrier (RFC) and proton coupled folate transporter (PCFT). Long-term oversupplementation, however, did not affect the transport.
Discussion and conclusion: Short-term folate oversupplementation leads to a significant decrease in intestinal absorption and renal reabsorption of folate in association with down-regulation of RFC and PCFT via some post-transcriptional mechanisms.

WS10 04
Factors Affecting 5-Methyltetrahydrofolate (5MTHF) Stability: Implications for 5MTHF Deficiency Observed in Inherited Metabolic Disorders

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Background: Deficiency of 5MTHF in CSF has been documented in a range of inherited metabolic disorders, including those where mitochondrial function is impaired.
Aim: The aim of this study was to gain further insight into the factors that influence 5MTHF stability in CSF by utilising a range of experimental model systems.
Methods: 5MTHF was quantified by reverse-phase HPLC with fluorescence detection. The stability of 5MTHF was determined in, (i) phosphate buffer (pH 7.4), (ii) pooled human CSF, (iii) phosphate buffer in the presence of ascorbate, (iv) phosphate buffer and (v) pooled human CSF in the presence of a hydroxyl radical generating system utilising Fenton chemistry ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$). All experiments were carried out in air saturated conditions at 37°C under timed incubations.
Results: 5MTHF (500 nM and 150 nM) degraded in buffer, with approximately 46% and 27% disappearing over time periods of 150 and 210 minutes, respectively. In contrast, endogenous 5MTHF present in CSF remained relatively stable. Addition of ascorbate (150 µM) stabilised 5MTHF in buffer. In both buffer and CSF, hydroxyl radical generation led to the rapid degradation of 5MTHF.
Discussion and conclusion: The relative stability of 5MTHF in CSF, in contrast to buffer, infers the presence of a protective factor counteracting degradation. The observation that ascorbate, which is present in CSF, confers 5MTHF stability, suggests that this is a biologically relevant protective factor. The presence of a hydroxyl radical generating system markedly increased degradation in CSF, indicating that any protective mechanism present may be overwhelmed. These findings suggest that oxidative stress within the CNS can lead to decreased 5-MTHF availability.

WS10 05
Folate-dependent regulation of cellular motility

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Cell motility is regulated by a complex network of extracellular signals including essential nutrients. One of such nutrients is folate, a vitamin serving as a coenzyme for the fundamental metabolic reactions of one-carbon transfer. Folate abundance is beneficial for cellular proliferation, which require efficient folate pathways to support de novo nucleotide biosynthesis and methylation processes. Not much is known about the role of folate in motility regulation.
We studied the role of folate in regulating cancer cell motility.
We evaluated effects of media folate on motility and actin dynamics in a cell culture model by several invasion/migration assays and fluorescence-based techniques. Using bioluminescent imaging upon tail vein injection of A549 cells stably expressing firefly luciferase, we monitored colonization of cancer cells in the lung of SCID mice kept on normal *vs.* folate deficient diet.
We have shown that folate deprivation strongly inhibits migration and invasion of cancer cells through a mechanism associated with robust dephosphorylation of the actin depolymerizing factor cofilin by two major cellular phosphatases, PP1 and PP2A, followed by the alterations in actin cytoskeleton. Upon folate withdrawal we observed F-actin stabilization, re-distribution of cytoplasmic actin toward strong preponderance of filamentous actin and formation of actin stress fibers. We have further demonstrated that the dietary folate restriction prevents colonization of cancer cells in lung of SCID mice.
We have demonstrated distinct intracellular pathways regulating motility in response to folate status. Our data further suggest that these mechanisms underlay folate effects in enhancing cancer-related metastatic processes.

WS10 06
Stable isotope dilution assays as tools for determining folate contents and bioavailability

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Introduction: Folates play a crucial role as coenzymes in the metabolism of one-carbon groups. Therefore, folate deficiency is considered to increase the risk of neural tube defects and is suspected of being associated with the development of several widespread diseases.
For examining folate concentrations, one of the most accurate quantitation method is the stable isotope dilution assay (SIDA), which is based on the use of isotopically labelled analogues of the analytes as internal standards. The merits of SIDA include the ideal compensation for losses and superior specificity.
Objective: To establish accurate dietary recommendations and to evaluate folate bioavailability, different approaches can be pursued. Of these, two short-term designs were applied.
Methods: In the first investigation, we examined bioavailability in a large-scale human study with application of 3 model foods and folic acid as the reference dose. Folates were quantified in the test foods and in the plasma of the volunteers by newly developed SIDAs. A second approach was a double label isotope study, in which deuterium and 13C-labels were used to differentiate oral doses from analytical internal standards.
Results: Calculation of area under the curve data of the post-dose plasma folate levels revealed a folate bioavailability of 20%, 73% and 33% in Camembert cheese, Spinach and wheat germs, respectively, relative to folic acid.
Using the double label technique, we were able to detect the signals of all folate isotopologues in blood plasma and to show that folate vitamers show different absorption behaviours.

Conclusion: As folate bioavalability widely varies for different foods and different vitamers, further human studies are necessary to compile valid dietary recommendations. Differently labelled folates are perfect tools for this task.

Advanced experimental models in vitamin research

WS11 01
New Focus on Pyridoxamine effects in Experimental Diabetic Nephropathy

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Diabetic nephropathy is a clinical syndrome characterized by marked decline in glomerular filtration rate (GFR),persistent Albuminuria and arterial hypertension in the absence of clinical or laboratory evidence of any other disease. It has become the main cause of end-stage renal failure (ESRF). Pyridoxamine is a vitamer in the vitamin B6 family which have certain metabolic effects. It can inhibits the maillard reaction and can block the formation of advanced glycation end products. Present work aimed mainly to study the metabolic effect of pyridoxamine in Diabetic nephropathy.
Method: Male Albino rats were rendered diabetics by alloxan administration 90 mg/kg body weight, Later division into 2 main groups. The first one has received pyridoxamine orally (180 mg/kg bw) daily for 6 weeks, the other one was kept as diabetic control. certain biomarkers were selected for evaluation of pyridoxamine effect.
Results: pyridoxamine induced significant decrease in serum glucose, fructosamine, urea, creatinine, CRP, urinary microalbumin, protein, renal MDA, expression of TNF-α, TGF-β-1 joined with increased GSH as compared to diabetic control group>
Histopathological examination of renal tissues illustrated certain regeneration attempts, dilated peritubular capillaries and normal appearance of renal tubules with focal interstitial lymphocytic aggregation
Conclusion: Marked improvements were observedin renal functions, antioxidant status, glycemic control. This may deserve the presentation of pyridoxamine as a new therapeutic candidates for diabetic nephropathy.

WS11 02
Dietary betaine supplementation enhances growth performance, carcass and immune response of New Zealand White rabbits under high ambient temperature

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A total of 120 weaned New Zealand White male rabbits, 6 weeks old, were randomly divided into five experimental treatments (24 each). Animals were fed *ad libitum* the basal diet supplemented with 0 (control), 250, 500, 750 and 1000 mg betaine /kg diet from 6 to 12 weeks of age. Animals were provided with water freely. The average daily temperature and relative humidity inside the rabbitry were 30.3±0.9 °C and 76.2±2.5 %, respectively. Under heat stress conditions, diet significantly increased the body weight and hot carcass weight and significantly reduced the feed conversion. Dietary 1000 and 750 mg betaine/kg increased (P<0.05) final body weights (2529.1 and 2418.5 g, respectively) compared with the control (2110.3 g). Betaine supplementation ameliorated some of the adverse effects of heat stress on immune responsiveness, rectal temperature and respiration rate. Dietary 250, 500, 750 and 1000 mg betaine/kg led to a decrease in rectal temperature (40.03, 39.85, 39.63 and 39.53 °C, respectively) compared with the control (40.20 °C). The inclusion of 1000 mg betaine/kg in the growing rabbits' diets nearly doubled the humoral and immune responses compared to the controls (P<0.05) and significantly reduced rectal temperature and respiration rate. Serum T3, T4, total protein, globulin and total lipids were significantly increased while serum glucose concentration was significantly decreased due to dietary betaine. In conclusion, supplemental dietary betaine enhanced growth performance and humoral and cell-mediated immunity as well as reduced rectal temperature and respiration rate in growing rabbits subjected to heat stress. From an economic point of view, high levels of betaine are not recommended because betaine is reasonably effective at lower, less expensive, doses.

WS11 03
Ameliorative Effects Of Beta Vulgaris (L.) On Ethanol – Mediated Hepatotoxicity And Oxidative Stress: In Vitro And In Vivo Studies

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Background: The roots of *Beta vulgaris* Linn. (Family - Chenopodiaceae) are widely used in Indian traditional medicine for various ailments including hepatic disorders[1,2].
Objective: The aim of present study was to investigate the protective effect of *Beta vulgaris* root (BV) on ethanol induced hepatotoxicity and oxidative stress using *in vitro* and *in vivo* screening models.
Methods: In the *in vitro* evaluation, primary cultures of rat hepatocytes were exposed to ethanol with or without various plant samples or silymarin for 2 h. The cell viability and the levels of serum marker enzymes were determined. In the *in vivo* evaluation, rats were treated with BV or silymarin once daily for 7 days and a single oral dose of ethanol. Histopathological changes and the levels of serum marker enzymes and antioxidants were assayed.
Result: In the *in vitro* evaluation, butanol fraction of BV (BVBF) was found to be more potent than others as evident from marked increase in cell viability and decrease in serum marker enzymes. In the *in vivo* evaluation, BVBF and silymarin elicited a significant hepatoprotective and antioxidant activity as evident by lowering the serum marker levels and elevation of antioxidants in a dose dependent manner. The biochemical observations were supplemented with histopathological results.
Discussion and conclusion: The present study demonstrates that butanol fraction of *Beta vulgaris* roots has significant hepatoprotective activity against ethanol induced hepatotoxicity, which could be attributed to the antioxidant effect of the constituents and enhanced antioxidant defenses.
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WS11 04
Methyl Donor Deficiency influences development, mucosal barrier, inflammation, and innate immunity in the Small Intestine of New Born Rats

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Background: Inflammatory bowel diseases (IBD) are a major public health problem. The potential link between methyl donors and IBD is supported by population studies. Methyl donor deficiency (MDD) aggravates experimental colitis in rats.
Aims: We investigated whether a diet lacking methyl donors (folate, vitamin B12 and choline) may affect development and functions of small intestine in rat pups from dams subjected to the deficiency during gestation and lactation.
Methods: Pathways related to development, mucosal barrier and innate immunity were studied in both proximal and distal intestine of rat pups.
Results: A global wall hypotrophy was observed in distal small bowel with increased crypt apoptosis, loss of enterocyte differentiation in villous and reduction of intestinal phosphatase alkaline production. Cleaved caspase-3 immunostaining and Apostain labelling index showed increased crypt apoptosis. Decreased proliferation was observed in crypts of the proximal small bowel with a reduced number of MCM-6 and PCNA-positive cells. This lack of enterocyte differentiation in distal small bowel was associated to impaired expression of β-catenin and decreased β-catenin/E-cadherin interaction. Low Protein Phosphatase 2A expression levels may trigger increased β-catenin degradation. MDD also affected proximal small bowel by decreasing Paneth cells number after immunostaining for lysosyme and by reducing goblet cells number and mucus production after immunostaining for mucin-2. Decreased expression of PPARgamma and hypomethylation of PGCalpha were consistent with the decreased expression of beta-Defensin1, a target of PPAR gamma.
Conclusion: The MDD produces dramatic effects on enterocytes, which may predispose to IBD

WS11 05
Creatine supplementation and homocysteine metabolism in rats fed choline-deficient diet

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Background: Choline-deficient diet (CDD) impairs methionine metabolism and increases homocysteine (Hcy) levels. Studies have shown that creatine supplementation modulates the flux of transmethylation reactions and decreases blood (Hcy) levels. However, the effects of creatine supplementation on CDD have been poorly explored.
Aim: To examine the effects of creatine supplementation on Hcy metabolism in rats fed choline-deficient diet.
Methods: Twenty four rats were divided into 3 groups of 8 rats each: control diet (C), choline-deficient diet (CDD), choline-deficient diet supplemented with creatine (Cr). The CDD diet was AIN-93 without the choline recommended content. Creatine was supplemented by the inclusion of 2% in the diet. The rats received the diets for 4 weeks.
Results: The CDD diet significantly increased Hcy concentration (50%) and decreased hepatic S-adenosylmethionine concentration (SAM) (25%). Creatine supplementation significantly increased plasma (303%) and liver (478%) creatine concentration and greatly down-regulated endogenous creatine synthesis, as evident from a ~80% decrease in renal arginine:glycine amidinotransferase activity. This mechanism caused reduction in plasma Hcy (C 11.2±0.7, CDD 16.9±1.3, Cr 10.1±0.6, umol/L). However, it did not returned hepatic SAM levels to normal. In addition, there were not changes in plasma cysteine, methionine, vitamin B12 and folate. It was also found increased plasma MDA (25%) and ALT (30%) levels. Creatine supplementation partially normalized these perturbations.
Conclusion: Creatine supplementation prevents increased Hcy induced by choline-deficient diet probably by methyl flux modulation.
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WS11 06
Homocysteine-lowering therapy: a novel non vitamin drug

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We recently observed that new chemical entities, obtained by grafting DG1 (a dithiolethione moiety) onto existing drugs, notwithstanding their deep chemical differences, shared a particular characteristic, affecting aminothiol metabolism in several tissues. We also found that a similar effect was obtained in animals treated with DG1 alone. Starting from these observations we had the idea to test the parent compound DG2 (DG1 is one of its metabolites) as possible lowering Hcys therapy. Our data obtained on rats essentially show that:

i) oral administration of DG2 significantly decreases total Hcys in normal rats;
ii) oral administration of DG2 significantly decrease total Hcys in hyperomocysteinemic rats;
iii) oral treatments with DG2 increase glutathione (GSH) and Cysteine (Cys) levels not only in blood but also in most of analysed tissues (in particular in liver and kidney):
iv) GSH (and Cys) increase were paralleled by their enhanced export in particular by liver and kidney; this, in turn, evokes a higher thiol to disulfide ratio in plasma and the consequent transformation of the majority of circulating homocysteine (protein bound) into low molecular weight form(s) that are more easily excretable or metabolizable to Cys.

It is noteworthy that GSH increase and Hcys decrease may have synergic action on preventing the detrimental actions of hHcys: GSH is a antioxidant compound and its increase may help to balance the damaging effects of Hcys according to oxidative stress hypothesis.
DG2 is a potent drug able to decrease circulating homocystene and warrants soon a study in humans in alternative to the classic vitamin therapy.

POSTER PRESENTATIONS

P1 – B12, biochemistry, transport, metabolism and disorders

P1 01
Metabolism and Disorders of Vitamin B_{12}

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Background: Vitamin B_{12} is not commonly thought of as causing deficiency as it is abundant in most people's diet in the form of animal proteins.
However, factors affecting its uptake, transport in the blood and storage in tissues include the presence and efficacy of hydrochloric acid, Intrinsic Factor, plasma transport and tissue storage proteins.
The conversion of homocysteine to methionine, important for methylation reactions, requires vitamin B_{12} and folic acid as co-factors. If either of these vitamins is lacking, homocysteine will accumulate in tissues.
Discussion: Other than pernicious anaemia health issues leading to lowered B_{12} status include cystic fibrosis, hydro- or achlorhydia and atrophic gastritis, gastric or ileal resection, and liver pathology. Deficiency symptoms usually develop over several years and can be difficult to diagnose initially.
As a result, homocysteine can accumulate and lead not only to heart disease but also to Down syndrome, spina bifida, neuropsychiatric disorders, osteoporosis and DNA damage.
Serum vitamin B_{12}, the most commonly employed blood test, fails to diagnose the deficiency accurately, leading to underdiagnosis of vitamin B_{12} deficiency. Measuring the metabolic intermediates methylmalonic acid and homocysteine, or holo-transobalamin II, are more accurate markers.
The best treatment option (other than for lack of B_{12} in the diet) is regular intramuscular vitamin B_{12}injections.
Conclusion: Vitamin B_{12}-related health disorders are often overlooked due to non-specific symptoms and lack of (accurate) testing. Treatment should be implemented in GIT pathologies to avoid the flow-on effects with major repercussions to life quality and costs to the health care system.

P1 02
Vitamin B_{12} – The Missing Link in Mental Health?

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Background: Nutritional repletion and optimization of nutrient status can avert or at least improve many health problems including mental health, thus reducing the burden on the national health care system. One such nutrient is vitamin B_{12} - a commonly overlooked factor. A literature search was conducted on the link between low vitamin B_{12} (with or without folate) status and neurological and psychological health problems.
Discussion: Both vitamin B_{12} and folate are required to lower homocysteine and are thus cofactors in methylation reactions. Low status of either of these vitamins can compromise methylation, potentially leading to a range of health problems, including psychological and neurological symptoms, and dementia.
Apart from lack in the diet (as in vegans) low vitamin B_{12} status is encountered frequently in people with gastrointestinal and liver pathology as this compromises the absorption and utilization of the vitamin. This may provide the missing link to the above-mentioned pathologies.
The most commonly employed test for vitamin B_{12} status is serum B_{12}, an unreliable marker of vitamin B_{12} status. Therefore, some people with low vitamin B_{12} status may go undiagnosed. Once symptoms appear, especially regarding dementia, repletion with the vitamin is unlikely to reverse existing neurocellular damage; hence it is prudent to identify and treat those at risk well before signs and symptoms develop.
Conclusion: As vitamin B_{12} is safe, affordable and non-toxic it should be provided to those at risk either orally on a daily basis, or administered intramuscularly in cases of compromised gastrointestinal or liver function, in order to maintain adequate body stores and reduce the risk of methylation-related pathology.

P1 03
Vitamin B12 Prodrugs for Chemotherapy

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Background: Vitamin B12 is an attractive molecule for chemotherapy for 2 reasons, its essential role in the growth of fast-growing cells and its ability to release the axial ligands inside the cells. The latter guarantees the liberation of anticancer drugs at tumor sites, if introduced at the axial position of B12. As a proof, the crystal structure of vitamin B12-{PtCl(NH3)2} conjugate (1), its physio-logical stability[1a], biological activity[1b] and cytotoxicity[1c] were previously reported.
Aim: This work studies the delivery capability of vitamin B12 to other non-metal chemotherapeutic agents including cytarabine, dacarbazine, and anastrazole.
Methods: HPLC, NMR, IR, and MS (characterization); MTT and Resazurin assays (IC50).
Results: All three compounds, B12-cytarabine (2), B12-dacarbazine (3) and B12-anastrazole (4) were stable in a mixture with human serum albumin for at least 3 days. IC50, μM=0.2 (2 on leukemia cancer K562 cells). Both anastrazole and (4) do not show IC50 (on breast cancer MCF7 cells). IC50 of (3) is under investigation.
Discussion and conclusion: The stability of B12-prodrugs in human serum albumin guarantees the availability of the compounds for tumor uptake. The in-vitro experiments were performed with no addition of B12 transport proteins. As B12-prodrugs enter the cells through receptor-mediated uptake, the prodrugs are expected to have higher cytotoxicity than the reported values in living organisms with sufficient expression of transport proteins. These results show that B12 is suitable to deliver different types of chemotherapeutic agents, metal-based (cisplatin) and non-metal (cytarabine, dacarbazine, and anastrazole), with different types of cancer (cytarabine for leukemia, dacarbazine for melanoma, and anastrazole for breast cancer). As anastrazole is efficient for breast cancer after surgery and for metastasis in pre- and post-menopausal women, it is not surprising that (4) and anastrazole show no IC50 in in-vitro assay for MCF7 cells. Therefore, it is interesting for a future in-vivo experiment with (4).
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P1 04
The Utility of Holotranscobalamin II as a Marker of Vitamin B12 Balance

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Question: Holotranscobalamin II (holo-TC II) is the complex binding of cobalamin or vitamin B12 (Cbl) with transcobalamin II, which carries Cbl into cells. A decrease in holo-TC II is the first sign of poor absorption and is the earliest marker of Cbl deficiency.
The question of the study was to highlight the value of testing holo-TC II as an early marker of B12 deficiency.

Methods: 119 adult patients with anaemia and neurological diseases were studied.
Fifty normal blood donors were used as controls.
Holo-TC II and Cbl serums were measured using MEIA and RA.
Results: In accordance with the results the patients were divided into 3 groups: Group A) 27 / 119 patients (22.69%) with decreased holo-TC II (< 50 pmol / L) and decreased Cbl (< 300pg/mL), Group B) 36/119 patients (30.24%) with decreased holo-TC II and normal Cbl and Group C) 56 / 119 patients (47.06%) with normal holo-TC II and normal Cbl.
Early detection of B12 deficiency is crucial to the prevention of neurological complications that may develop even in the absence of macrocytic anaemia. Holo-TC II measurement reflects the level of Cbl tissue. In the patients studied 30.25% showed decreased levels of holo-TC II with normal Cbl serum, which would be considered normal using only B12 doping. Depletion of holo-TC II is the first sign of B12 deficiency.
Conclusions: When vitamin B12 deficiency is suspected, holo-TC II should also be tested. Measuring holo-TC II is useful for predicting early B12 depletion.
Testing holo-TC II reflects B12 absorption and partially replaces the Schilling test.

P1 05– WITHDRAWN

P1 06
Plasma 25-(OH) vitamin D levels in the patients with Parkinson syndrome

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Background: There is some evidence suggesting that patients with Parkinson syndrome (PS) fall and break a bone easily when compared to age-matched healthy subjects. It is unclear what the patients with PS affected.
Aim: The aim of this study was to investigate the association with plasma 25-(OH) vitamin D (VitD) levels and neurological symptoms in the patients with PS.
Methods: The subjects constructed 36 patients with Parkinson's disease/dementia with lewy bodies (PD/DLB; 20 females, average age 72.2 years), 25 patients progressive supranuclear palsy/corticobasal degeneration (PSP/CBD; 10 females, average age 75.2 years) admitted to our hospital. Twenty one normal healthy volunteers (CTL) composed the control group (13 females, average age 76.1 years). We evaluated gender, age, period of illness, stage of the Hoehn-Yahr stage (H-Y), orthostatic dysregulation, dementia, hallucination, wearing-off, serum albumin and hemoglobin, 123I-MIBG cardiac uptake and levodopa equivalent dose. We examined a statistical analysis by the multiple linear regression.
Results: VitD levels in plasma were 16.3 +/- 5.0 ng/mL (mean +/- S.D.) in the patients with PD/DLB, 14.3 +/- 5.9 ng/mL in the patients with PSP/CBD and 25.5 +/- 6.8 ng/mL in CTL. VitD levels in the patients with PD/DLB and PSP/CBD were significantly lower than in the CTL ($p < 0.0001$). The final linear registration model included not only H-Y (regression coefficient -0.384; $p = 0.0024$) but also serum albumin (regression coefficient 0.358; $p = 0.0043$) as significant variable influencing the VitD ($R = 0.659$, $p < 0.0001$).
Discussion and Conclusion: VitD is one of vitamins associated with the metabolism of a calcium. Our results suggest that the falling and fracture easily in the patients with PS associate with not only motor dysfunction with illness but also the subjects of nutrition include VitD.

P2 – B-Vitamin supplementation

P2 01
More Dietary Biotin Is Needed When Diet of Quails Contains Soybean Oil

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Background: Biotin is involved in fatty acid synthesis and is also associated with certain diseases such as diabetes mellitus, liver disorders, and immunological abnormalities.
Aim: The objective of this study was to evaluate the effects of dietary biotin supplementation with different fat sources (soybean oil or beef tallow) on live performance, carcass characteristics, organ weights, and plasma concentrations of some minerals and metabolites in Japanese quails.
Methods: One hundred and twenty 10-day-old healthy Japanese quails were assigned to 4 treatment groups. The experiment was designed in a 2 X 2 factorial arrangement using two types of fat source (5% soybean oil or 5% tallow) and two levels of biotin supplements (0 or 300 mcg/kg diet).
Results: Feed intake was not influenced by either fat source or biotin supplementation in the diet ($P > 0.05$). Final body weights and feed efficiency were greater when the diet of quails contained soybean oil compared with that of tallow ($P \leq 0.012$). Liver weights ($P = 0.017$) and abdominal fat accumulation (although not significantly, $P = 0.17$) were greater in quails fed tallow. Plasma cholesterol and triglyceride concentrations decreased with soybean oil feeding, but cholesterol concentrations increased with biotin supplementation. Biotin supplementation increased plasma glucose concentrations ($P = 0.01$).
Discussion and Conclusion: It was concluded that biotin can be supplemented at 300 mcg/kg diet to the quail diets containing %5 soybean oil.

P2 02
Supplementation with zeolite and vitamins B_1, B_2 and B_6 – *in vitro* experiment

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Preparations on the basis of the zeolites are used for adsorption of toxic substances of organic and nonorganic background and they find more and more use in the veterinary and human medicine and pharmacy.
The aim was to estimate possibilities of zeolite to adsorB-vitamins B_1, B_2 and B_6 in acid and neutral solution, as well as the nature of the process (saturability and reversibility).
For vitamins B_1, B_2 and B_6 detection the HPLC method with fluorescent detector was used as a specific and sensitive enough. Analite separation and detection were carried out by applying reverse-phase method on the C18 column, and fluorescent detector with variable wavelength, respectively. Examination of vitamin solutions in concentrations 1, 2 and 5 mg / L, were carried out at pH=2 and pH=7, at 37 ° C, and the determination of vitamine content in the presence of zeolite was done before and 10, 30, 60 and 180 minutes after adding zeolite in the vitamine solution. Ion competitiveness was examined by adding commercial feed mixture (grower) which contained defined content of examined vitamines in the pH=2 and pH=7 zeolite solution.

Zeolite significantly adsorbs vitamins B_1, B_2 and B_6 in the acid and neutral solution at 37 ° C already in the first ten minutes of the contact. Adsorption is irreversible in single solution, and reversible after changing of solution pH from acid to neutral. In neutral solution significant ions competition for zeolite adsorption exists, so there no statistically significant vitamins B_1, B_2 and B_6
Adsorption occurs, while in an acid solution competition is smaller and zeolite significantly adsorbs these vitamins, although in less degree than under concurent ions absence conditions.

P2 03
Vitamin B(12) with folic acid ameliorates arsenic-induced liver damage via DNA protection by antioxidant systems

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The present study elucidated the protective role of vitamin B(12) with folic acid against arsenic-induced hepatotoxicity in female rats. Ingestion of sodium-arsenite- contaminated water [0.4 ppm/100 g body weight (b.w.)/day] in combination with vitamin B(12) plus folic acid (0.07 and 4.0 µg, respectively/100 g b.w./day) for 24 days to Wistar rats offered a significant protection against alone arsenic-induced distorted liver function, damaged histoarchitecture, elevated oxidative stress and DNA fragmentation. Arsenic only exposure decreased hepatic superoxide dismutase (SOD), catalase activities and the level of nonprotein-soluble thiol (NPSH) with a concomitant increase in thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CDs) in the liver. Our earlier studies on human blood samples from individuals (exposed to ≥ 100 µg/L As, ≥ 10 years) from few affected villages in Eastern India explore that more than 70% developed pigmentation and palmoplantar hyperkeratosis with many of those developing carcinomas. A significant decrease in antioxidant component like catalase, soluble thiol and recently recognized uric acid worsen the situation by generating free radicals as observed in significant rise in malondialdehyde ending up with increase in DNA fragmentation in WBC and arsenic associated mutagenesis carcinogenesis. To search for a therapeutic agent, in our rat experiment, it was observed that vitamin supplementation restrained the increase of TBARS and CDs by restoring catalase, SOD, and NPSH levels. Restricted generation of free radicals may be correlated to the protection of DNA stability and tissue morphology. This study explains the decisive role of vitamin B(12) with folic acid in amelioration of arsenic-mediated liver injuries. # **Correspondence:** Dr Smarajit Maiti, Associate Professor and Head, email: maitism@rediffmail.com

P2 04
Evaluation of the impact of a lipoic acid - based nutritional supplement on the serum paraoxonase activities in post-acute stroke patients undergoing rehabilitation

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Background: Paraoxonase 1 (PON1) is a high density lipoprotein (HDL)-associated protein responsible for the antioxidant properties of these particles.
Aim: To evaluate the dynamic of serum paraoxonase activities in post-acute stroke patients treated with a lipoic acid - based nutritional supplement.
Methods: We enrolled 28 post-acute stroke patients hospitalized for a period of two weeks for specific rehabilitation procedures. They were organized in two groups: group 1 (14 patients, 7 females/7 males, mean age 67.07 ± 2.90 y.o.) and group 2 (14 patients, 7 females/7 males, mean age 67.14 ± 2.85 y.o.). There was no statistical difference between these groups in respect to the medication. Patients from group 2 received a dose of 2 tablets/day of the nutritional supplement (ALAnerv®). Blood samples were taken at the hospitalization and at the discharge moments. We evaluated serum PON1 activities with three different substrates paraoxon, phenyl acetate and dihydrocoumarin, using previously described methods. In both groups we assessed the percentage of variation of the enzymatic activity between the hospitalization and the discharge moments. The non-parametric test Mann-Whitney was used for statistical analysis ($P < 0.05$).
Results: The percentages of variation for the enzymatic activities were compared between group 1 versus group 2: (i) paraoxon (9.73 ± 6.07 vs. -8,07 ± 5.84, $P = 0,019$), (ii) phenyl acetate (147.60 ± 121.04 vs. 18.39 ± 9.34, $P > 0.05$) and (iii) dihydrocoumarin (111.00 ± 122.85 vs. 118.70 ± 28.75, $P < 0.001$).
Discussion and Conclusion: The results of this pilot study suggest that administration of ALAnerv® was associated with significant variation of the enzymatic activities in respect to paraoxon and dihydrocoumarin. The data point the need to evaluate the effects of this nutritional supplement over a longer period of time.

P2 05
Evaluation of the effect of a lipoic acid - based nutritional supplement on the dynamic of the blood redox status in post-acute stroke patients undergoing rehabilitation

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Background: Oxidative and nitrosative stress are key elements in stroke etiology.
Aim: To evaluate the dynamic of the blood redox status in post-acute stroke patients treated with a lipoic acid-based nutritional supplement.
Methods: 28 post-acute stroke patients were hospitalized for specific rehabilitation procedures for a period of two weeks. The patients were organized in two groups: group 1 (14 patients, 7 females/7 males, mean age 67.07±2.90 y.o.) and group 2 (14 patients, 7 females/7 males, mean age 67.14±2.85 y.o.). There was no statistical difference between these groups in respect to the medication. Patients from group 2 received a dose of 2 tablets/day of the nutritional supplement (ALAnerv®). Blood samples were taken at the hospitalization and at the discharge moments. The following markers of oxidative stress

were assessed, according to previously described methods: total antioxidant capacity, total thiols, non-proteic thiols, protein carbonyls, oxidized low-density lipoprotein (LDL) particles (LDLox), lipid hydroperoxides and the activity of g-glutamyltranspeptidase. We calculated the percentage of variation for these markers between the hospitalization and the discharge moments. The non-parametric test Mann-Whitney was used for statistical analysis ($P < 0.05$).
Results: When comparing the percentages of variation between the two groups, statistically significant differences were obtained only for LDLox (-4.00 ± 6.08 vs. -78.31 ± 1.89, $P < 0.001$) and lipid hydroperoxides (36.52 ± 31.60 vs. -33.82 ± 12.20, $P = 0.029$).
Discussion and Conclusion: These preliminary results suggest that administration of ALAnerv® would contribute to the improvement of some oxidative stress parameters during the rehabilitation period. This study points the need to evaluate the effects of this nutritional supplement over longer time periods and in high dosages.

P2 06
Folic acid supplementation Effects in Patients with Type 2 Diabetes Mellitus

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Introduction: This study performed to determine the effects of supplementation of folate on indices of glycemic control, insulin resistance and lipid profile in men with type 2 diabetes under metformin (at least 1500mg daily) treatment.
Materials and Methods: This was a double-blind randomized controlled clinical trial. 68 men with type 2 diabetes participated with written consents. Patients were randomly divided in two groups; folic acid 5mg/day and placebo. All the patients received the tablets for 8 weeks. Anthropometric and nutrient intakes data obtained from each patient. Baseline and 8th week fasting blood glucose, HbA1C, serum insulin, insulin resistance, serum total cholestrol, TG, LDL-C, HDL-C, serum folate and plasma homocysteine were measured.
Results: Supplementation with folic acid for 8 weeks led to decrease HbA1C (P=0.019) and fasting blood glucose (P=0.006), serum insulin (P=0.028), insulin resistance (P=0.043) and plasma homocysteine (P<0.001), increase serum folate (P<0.001) of folic acid group. Conclusion: Pharmacological dose of folic acid supplementation for patients with type 2 diabetes decreased plasma level of homocysteine and improved glycemic control, insulin resistance and serum folate levels.
Key Words: Folic acid, Glycemic control, Insulin resistance, metformin, type 2 diabetes

P2 07
B-vitamins, Homocysteine And Anemia In Dialysis Patients

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Introduction: Many years ago it has been discovered that vitamin B therapy improved anemia in vitamin B depleted patients. Hemodialysis patients often show vitamin B deficiency. Vitamin B therapy should improve anemia in these patients, but until now there are contrasting data. Hyperhomocysteinemia, a risk factor of vascular and bone disease, is frequently detected in vitamin B poor hemodialysis patients. Animal models hypothesize that high homocysteine levels, giving a low methylation potential, may lower erythropoietin synthesis. Actually, we do not know whether homocysteine may interact with erythropoiesis.
Aim and Methods: In view of these considerations, we studied the effects of vitamin B therapy on homocysteine, hemoglobin, erythropoiesis stimulating agents (ESA) doses and erythropoiesis stimulating agents resistivity index (ESA RI) of hemodialysis patients. We performed a long, retrospective study. Hemoglobin values and ESA doses were assessed every month; homocysteine, folate and vitamin B12 values were measured every 4 months. We considered suitable for the study vitamin B treated patients without residual renal function, hemorrhagic accidents and blood transfusions during 2-years follow up. 33 patients were split by 3 groups, according to the mean homocysteine level: group A with normal homocysteine level (below 15 micromoles/litre), group B with moderate hyperhomocysteinemia (mean homocysteine level between 15 and 30 micromoles/litre) and group C with severe hyperhomocysteinemia (mean homocysteine level higher than 30 micromoles/litre).
Results: Group C patients had both higher hemoglobin levels and lower ESA RI than patients of group A and B (respectively for hemoglobin and ESA RI: 12,0 vs.10,9 vs. 11,4 g/dl - p< 0,05; and 4,2 vs. 13,3 vs. 11,6 - p< 0,05). Furthermore ESA RI was inversely associated to vitamin B12 levels (r = 0,44 – F= 7,5 – p< 0,05), suggesting a low consumption of ESA in B12 replete patients.
Conclusions: Hemodialysis patients with severe hyperhomocysteinemia had lower ESA RI than patients with normal or moderately high homocysteine levels. We think that homocysteinilation may up-regulate erythropoietin receptor stimulated by ESA. Severe hyperhomocysteinemia seems also to increase platelet count. High B12 levels are also associated to low ESA RI.

P2 08– WITHDRAWN

P2 09
Cardiovascular Risk Of An Elderly, Black South African Population In Sharpeville, South Africa

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Cardiovascular disease exists in epidemic proportions in developed countries and is an increasing problem in the developing world. Black elderly people in South Africa live under poor living conditions, this often results in house hold food insecurity and malnutrition, which has negative effects on the health status of the elderly including chronic disease of lifestyle.
The aim of this part of the study is to evaluate the baseline status of the cardiovascular risk markers as part of a multi-micronutrient programme to address malnutrition among elderly attending a day care centre.
This is an ethically approved, experimental study in 350 purposively selected samples of all the elders attending a day-care centre in Sharpeville, Gauteng, South Africa. Blood collection and analysis parameters were performed according to standard laboratory protocol in order to comply with SANAS accreditation requirement.
A prevalence of 68% hypertension and 27.9% obesity were identified as health risk markers. Biochemical blood markers indicated 32.5% females and 20.8% respondents had a decreased serum folate level,

vitamin B12 deficiency was detected in 29.2% males and 10.5% females. A very high fibrinogen level (5.3±2.2g/l) indicated a risk for cardiovascular disease.
It is concluded that this population is at high risk for cardiovascular disease. It is necessary to design an acute intervention to reduce the risk in this vulnerable population. It is proposed that a homocysteine lowering strategy (vitamin B6, B12 and folate supplementation) should be implemented and evaluated for its effect on the homocysteine metabolism, coagulation, fibrinolysis, inflammatory response and hypertension.

P3 – B-vitamins and choline in age associated diseases

P3 01
The relationship between serum biotin and oxidant/ antioxidant activities in bovinelameness

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Serum biotin concentrations, erythrocyte superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), reduced glutathione (GSH) and plasma thiobarbituric acid reactive substances (TBARS) were measured in 36 dairy cows, 18 of them were healthy and served as control. In the 18 cows with lameness problems, there were 5 cows with interdigital necrobacillosis, 5 cows with subsolar abscessation, 2 cows with solar ulcers, 2 cows with white line disease, 2 cows with chronic laminitis and 2 cows with septic arthritis. The degree of lameness was estimated to be slight in 3 cows, moderate in 11 cows and severe in 4 cows. Plasma fibrinogen levels and TBARS concentrations were increased significantly ($P< 0.05$) in lame cows compared to control group. The antioxidant enzymes GSH-Px, and CAT concentrations were increased significantly ($P < 0.05$) in lame cows. The level of reduced glutathione and the activity of SOD were significantly decreased in affected cows compared to healthy ones. Serum biotin levels in healthy cows ranged from 2.25 to 3.5 ng/ml while in lame cows, biotin levels ranged from 1.17 to 2.3 ng/ml. Biotin levels correlated positively with blood GSH ($r = 0.870$, $P < 0.05$), ($r = 0.735$, $P < 0.05$) and with GSH-Px ($r = 0.539$, $P < 0.05$) and with SOD ($r = 0.637$, $P < 0.05$), ($r = 0.449$, $P < 0.05$), ($r = 0.585$, $P < 0.05$) in both healthy and lameness affected subjects, respectively.

P3 02
Effect of folic acid injestion on homocysteine related patient comorbidities in U.S. Veterans

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Background: Exposure of vascular smooth muscle cells to homocysteine prior to cytokine stimulation ultimately leads to inflammation.
Aim: We aim to investigate comorbidities asspociated with vasculopathy in veterans with homocysteine above and below 13 umol/L on and off folic acid.
Methods: Relative risk calculations comapring homocysteine associated comorbidities on and off vitamins in a population of 278 veterans whose homocusteine levels were taken between 6/16/2009 and 8/06/2010.
Results: For patients with homocuysteine > 13, those taking folic acid supplementation had a significantly higher rate of diabetes than patients not takingfolic acid, RR=2.34 (95% CI: 1.12-4.89). For patients with homocysteine > 13, those taking folic acid supplements had a significantly higher rate of hyperlipidemia compared to patients with homocysteine > 13 not taking folic acid in this period, RR - 1.52 (95% CI: 1.06-2.16).Among patients not taking folic acid supplements, only those with low homocysteine levels had significantly lower incidence of CVAs, RR =0.217 (95% CI: 0.063-0.749), kidney disease, RR=0.217 (95% CI: 0.085-0.558), anemia, RR - 0.406 (95% CI: 0.172-0.955), and hypertension, RR=0.780, (95% CI: 0.627-0.97). Among psoriatics, those taking folic acid supplements had a higher incidence of diabetes mellitus, RR=2.353 (95% CI: 1.14-7.02).
Discussion and Conclusions: Taking folic acid doses of up to 1 mg daily may sometimes be associated with an increases risk of multiple disorders associated with vascular pathology, especially when a patient has elevated homocysteine.

P3 03
Postnatal Effects Of An Early Methyl Donor Deficiency On The Rat Brain: Specific Sensitivities According To Brain Structures

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Background: Folate and vitamin B12 are methyl donor precursors for the production of S-adenosylmethionine which plays a modulating role in disease occurrence. In particular they are involved in epigenetic/epigenomic mechanisms. Gestational and perinatal methyl donor deficiency (MDD) has early and late consequences yet incompletely elucidated.
Aim: We studied the effects of an early methyl donor deficiency on brain maturation and plasticity in rat pups.
Methods: Dams received a standard diet or a MDD diet (B12, folate, and choline) one month before mating. The regimen was maintained until weaning of the offspring (21 days), and replaced subsequently by a normal diet.
Results: At 21 days of age, the MDD diet produced an accumulation of homocysteine (hcy) in specific brain structures but results differed according to the concerned brain areas. The MDD diet was associated with histological impairments. Hcy accumulation in the hippocampus and the cerebellum was produced in part by a decreased expression of cystathionine beta-synthase (CBS) and methionine synthase (MS) only in the hippocampus. Neuroplasticity Proteins expressions were decreased in both brain structures. In parallel, behavioral tests showed an impaired maturation of functions related to cerebellum and hippocampus in case of deficiency. At 450 days of age, in spite of the return to normal nutritional conditions at weaning and recovery of metabolic markers, the deleterious tissular consequences of early exposure to deficiency lead to persistent functional disorders.
Discussion and Conclusion: The key mechanisms involved would occur at critical periods during the maturation of the various brain structures, thus highlighting the role of fetal programming.

P3 04
Perinatal Choline Supplementation Increases Neurotrophin Levels and Expression of Cholinergic Markers in the Hippocampus and Septum of an Alzheimer's Disease Model

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Background: Progressive synaptic dysfunction and neurodegeneration may be the result of accumulating levels of ß-amyloid protein (Aß) in Alzheimer's disease (AD). Overproduction of Aß has been shown to disrupt adult neurogenesis, reduce survival of newborn neurons, alter neurotrophin availability, and impair signaling pathways associated with learning and memory. Choline is an essential nutrient critical for brain development. In rats, supplementation of the maternal diet with choline has been shown to improve memory and cause biochemical changes in the brains of the offspring.
Aim: We propose to use perinatal dietary manipulation of choline as a means to elevate neurotrophin expression, improve rates of neurogenesis, and augment cholinergic neurotransmission in an AD mouse model.
Methods: Wildtype (WT) female mice were placed on either a control or choline supplemented diet then crossed with APPswe/PS1dE9 transgenic male mice. All pups were weaned to a control diet.
Results: We found that perinatal choline supplementation alters the expression of several trophic factors including nerve growth factor (NGF) in the hippocampus of both WT and APPswe/PS1dE9 mice, suggesting that choline supplementation enhances the trophic microenvironment in the brain that may protect against AD-associated neuronal insults. Moreover, protein levels of tyrosine kinase receptor A and the low-affinity NGF receptor p75 were significantly higher in choline-supplemented mice, which may render the cholinergic neurons more responsive to NGF signaling. Choline supplementation also prevented a decrease in CHAT protein in 9-month old APPswe/PS1dE9 mice.
Conclusion: This study may help develop a potential means of preventing or slowing the progression of AD.

P3 05
Perinatal Choline Supplementation Improves Social Behavioral Deficits in an Animal Model of Autism

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Background: Autism is a developmental disorder characterized by severe deficits in social interaction skills, impairments in verbal and nonverbal communication, and stereotyped, repetitive patterns of behaviors and interests. Prenatal exposure of rats to valproic acid (VPA) causes many anatomical and behavioral abnormalities similar to those seen in autistic patients. Choline is an essential nutrient critical for brain development. When rat maternal diets were supplemented with choline during embryonic day (E) 11-17, the offspring had improved memory and attention.
Aim: The purpose of this study was to evaluate the effectiveness of perinatal choline supplementation on ameliorating social behavioral deficits in an animal model of autism.
Methods: On E11, pregnant Wistar dams were given either a control or choline supplemented diet. On E12.5, rats received an ip injection of either saline or VPA (550 mg/kg).
Results: As expected, VPA delayed eye opening and decreased postnatal body and brain weights. Choline supplementation prevented the delay in eye opening and reduction in brain weight. Perinatal choline supplementation dramatically improved behavioral deficits in social interaction in both adolescence and adulthood. Control animals exposed to VPA displayed reduced time spent engaged in social interaction with either a familiar or unfamiliar rat and significantly more time in the center of the testing area, whereas choline supplementation increased time engaged in social interaction.
Conclusions: The results suggest that the perinatal nutritional manipulation of choline can counteract the behavioral alterations in VPA rats and reveal an important discovery for the treatment or possibly prevention of autism spectrum disorders.

P3 06
In vitro and *in vivo* inhibition of acetylcholinesterase by some anaesthetic drugs

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Acetylcholinesterase (AChE; EC 3.1.1.7) activities are usually measured in whole blood and the most commonly used substrate is acetylthiocholine. Acetylcholine is critical for an adequately functioning memory, and it is the subject of the majority of research looking for treatments for memory defects, like those found in Alzheimer's disease. Any mental health issue that involves memory or lack thereof, directly or indirectly relates to acetylcholine [1]. Current therapeutic strategies for the symptomatic treatment of Alzheimer's disease and other related disorders such as vascular dementia and dementia with Lewy bodies are aimed at enhancing the associated cholinergic deficit by inhibiting acetylcholinesterase (AChE) [2] resulting in a boost in endogenous level of acetylcholine (ACh) in the brain and an improvement of the cognitive function [1-3]. We report here the inhibitory capacities of lidocaine, articaine, bupivacaine against the AChE ezymes from human and the arthropod (*Drosophila melanogaster*) (AChE). The ezymes showed quite diverse inhibition profiles with these drugs. Inhibitory capacities of lidocaine, articaine, bupivacaine on hAChE and *Dm*AChE activity were determined using DTNB under *in vitro* conditions. IC_{50} values were determined to be in low micromolar ranges indicating that these drugs inhibit the enzymes quite effectively.
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P3 07
Motor function of 5-6 year old children who were exposed to Thiamine deficiency during infancy

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Background: Nutrient insufficiency can have irreversible effects on the rapidly developing brain, depending on the timing, dosage and duration of exposure. Towards the end of2003 agroup of infants were hospitalized, after being fed with a soy-based formula lacking in thiamine. Knowledge of the effects and long term implications in children exposed to thiamine deficiency is rare. This study aimed to investigate

whether children who had been exposed to thiamine deficiency during infancy, display motor difficulties at the age of 5-6 years.
Methods: In this case-control study, 39 children aged 5-6 years, who had been exposed to a thiamine deficient formula during infancy, were compared with 30 children matched for age and gender who had been fed with other substitute formulae. The motor function of the participants was evaluated by two known motor skill assessments: The Movement Assessment Battery for Children (M-ABC) and the Zuk Assessment for Motor Function and Movement Skills.
Results: Both assessments showed significant differences between the study and the control groups for gross and fine motor function ($p< 0.001$, except for ball skillsp= 0.01) and grapho-motor function ($p= 0.004$). The most significant difference was noted in balance control functioning ($p< 0.001$). In the study group, both assessments (M-ABC, Zuk) concurred on the high rate of children suffering from Developmental Coordination Disorder (DCD) in comparison to the control group (M-ABC: 56% vs. 10%, Zuk: 59% vs. 3%,$p< 0.001$).
Conclusions: Thiamine deficiency in infancy affects gross and fine motor function in childhood. The current study emphasizes the crucial role thiamine takes in normal motor development and it's possible effect on formation of brain structures.

P3 08
B-vitamin deficiency and the Blood Brain Barrier in a mouse model of Alzheimer's Disease

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The role of B-vitamins and high homocysteine (HCY) levels in the Late Onset Alzheimer's Disease (LOAD) is a controversial topic. We previously applied a nutritional model of B-vitamin deficiency to a transgenic mouse model of Alzheimer's Disease to study the influence on Alzheimer-like features. The deficiency of Folate, vitamin B12 and vitamin B6 primarily resulted in increased plasma HCY. Consequently, tg mice under B-vitamin deficiency showed increased Aβ overproduction due to the impairment of DNA methylation and PSEN1 and BACE1 overexpression.
We hypothesized that Aβ scavenging and transportation could also be deregulated by B-vitamin deficiency and decided to investigate Aβ passage through the Blood Brain Barrier (BBB). The idea of studying the role of B-vitamins in the BBB functions finds a rationale also in the theories postulating a common basis for LOAD and dementia of vascular origin (Vascular Dementia: VaD), since B-vitamins and HCY are common risk factors.
Aβ transport across the BBB was analyzed through the study of the levels of its receptors RAGE, which is responsible for the passage of Aβ from the bloodstream to the brain tissue, and LRP1, which is responsible for the inverse passage. Endothelial dysfunction was studied by immunohistochemistry.
Our results indicated that RAGE is more expressed and LRP1 less expressed in tg mice. B-vitamin deficiency induces RAGE increase in the frontal and parietal microvasculature and intracellular RAGE decrease and LRP1 increase both in Tg and wt mice within frontotemporal, parietal cortex and hippocampus. Preliminary analysis revealed different response of cerebral and systemic vasculature to B-vitamin deficiency.
These results evidence that B-vitamin deficiency can modulate RAGE and LRP1 expression and endothelial function with possible consequences for Aβ transport across the BBB and for its clearance at the level of CNS cells.

P3 09
Dietary intake does not predict vitamin B12 status in Chilean elderly

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Background: Low vitamin B12 (B12) status is prevalent among elderly and usually attributed to its poor absorption from food, but few studies have examined the extent to which it is predicted by low intake of the vitamin in this age group.
Aim: To evaluate the relationship between B12 intake and status biomarkers in free-living, healthy Chilean elderly recruited from the community.
Methods: Serum B12, total homocysteine (tHcy), methyl malonic acid (MMA), holo-transcobalamin (holo-TC), serum folate (SF) and B12 intakes were assessed at baseline (n=282) in a RCT of B12 supplementation in Chilean elderly.
Results: 48% of subjects were B12 deficient (serum B12 <148 pmol/L). The main sources of B12 were meat (44%), dairy products (22%) and B12-fortified milk delivered by Chile's Ministry of Health (18%). Total B12 intake was 2.9±1.2 µg with 66% reaching the EAR (2.0 µg/d). Serum B12 was 152 (94-255) pmol/L, tHcy 12 (10-15) nmol/L, MMA 228 (147-392) nmol/L, holo-TC 48.1 (34.7-67.4) pmol/L and SF 12.0 (8.0-17.0) ng/mL. Neither total dietary B12 nor tertiles of intake were correlated with any biomarker.
Conclusions: Meat, dairy products and milk fortified with B-12 were the main sources of B12, but providing fortified milk (1.4 µg/d B12) was insufficient to achieve normal status. The high prevalence of vitamin B12 deficiency and depletion in this elderly population was not predicted by dietary vitaminB12 intake.
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P3 11
Low holotranscobalamin and cobalamins predict incident fractures in elderly men – The MrOS Sweden study

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Question: Whether cobalamins, serum holotranscobalamin, serum folate and plasma total homocysteine are associated with risk of incidental fracture in older men.
Methods: The MrOS study is a population based study of 790 elderly men in the Gothenburg, (median age of 75.3), without ongoing B-vitamin medication. Baseline data included physical performance, falls, BMI, BMD, cobalamins, holotranscobalamin, folate and homocysteine and incidental fractures were registered. Men were followed for up to 7.4 years (average 5.9 years).
Results: During follow up 110 men sustained one or more fracture. Overall, the risk of fracture multivariable adjusted increased by 39% and 26% for each SD increase in cobalamins and holotranscobalamin, respectively (HR 1.39; 95% confidence interval 1.12-1.73 and HR 1.26; 95% confidence interval 1.03-1.55). Men in the lowest quartile of cobalamins and holotranscobalamin, had an increased risk of fracture, (HR=1.70, 95% CI 1.13-2.56 and HR=1.70, 95% CI 1.14-2.54, respectively) than did those in quartile 2-4. Corresponding figures for vertebral fractures were HR 2.47 (1.304-4.57) and 2.49 (1.36-4.57) respectively. Poisson regression models demonstrated that both cobalamins and holotranscobalamin below the median (310 pmol/L and 51.8 pmol/L, respectively) was inversely related to yearly incidence of fractures. No associations between folate, homocysteine and incident fractures were seen.
Conclusions: Older men with low holotranscobalamin and cobalamins have an increased risk of incident fractures. This association does not seem to be mediated via BMD and other known risk factors for fracture.

P4 - B-vitamins and choline in birth defects

P4 01
Folic acid and Grape Seed Extract Prevent Azathioprine induced- fetal malformations and Renal Toxicity in Rats

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Azathioprine (Aza) is an important drug commonly used in the therapy of the autoimmune system disorders . It induces many hazard effects that restrict its use. The effects of administration of grape seed extract (GSE) and folic acid on Aza toxicity by gavage simultaneously for 4 weeks were studied by determining the changes in kidney histology,the glutathione level (GSH), and lipid peroxidation content as malondialdehyde (MDA) in the kidney tissue were measured. Additionally, their effects on the fetal development were investigated. Aza induced a renal damage as indicated from the pronounced changes in histological structure, a significant increase in serum urea and creatinine, and MDA content in the kidney tissue. Meanwhile, the GSH activity was significantly decreased. Co-treatment with GSE significantly minimised the previously mentioned the hazard effects of Aza by ameliorating the antioxidant activity. At this point folic acid induced a non significant protective activity. The results also revealed that administration of folic acid and GSE at 6th through 15th day of gestation did not altered fetal development. While, Aza administration clearly disturbed fetal development as indicated from abnormal feto-maternal attachement and a significant decrease in fetal weights and numbers. Furthermore, co-administration of both drugs significantly minimised simillary the hazards of Aza on the fetal development. It may be concluded that GSE and folic acid are a useful herbal remedies, especially for controlling oxidative damages and are considered as a protective agent against Aza toxicity.

P5 – Choline & Betaine

P5 01
Linear and logistic model in meta-analysis of milk yield responses to rumen protected choline supplementation

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Choline requirements for lactating dairy cows have not been established. In spite of that, higher choline availability (by feeding rumen-protected choline, RPC) can improve milk production, suggesting that this nutrient may be limiting either for lactating dairy cow, or for the transition cows in the late gestation and in early lactation. Based on these assumptions, we investigated the effects of rumen protected choline administration on milk yield in 12 different studies, carried out between 1991 and 2008. Accordingly, 42 experimental groups for milk yield, have been considered in the dataset. Mean and standard error data have been tested with both linear and logistic regression models and milk yield response to RPC supplementation has been investigated. Dataset analysis indicated that although most of variability among experiments was related to treatments schedule, dry matter intake and dietary composition, these factors were also highly correlated. For this reason, and in order to avoid any redundancy, our regression analysis included only RPC supplementation (control/treated) as fixed effect, while all other variables have been considered as experimental effects and treated as random components in both considered models, in order to take into account the difference among experimental conditions. The results of this analysis indicate that the linear model that assume a constant RPC response and utilization efficiency appears less appropriate, if compared to the logistic one, especially when high choline (> 20g/d) supplementation is considered.

P5 02
New substrates for the transporters of the CTL (choline transporter like) gene family

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The first CTL family members to be characterized were CTL1 and CTL2, and the expression of both of these proteins correlates with changes in the level of choline uptake. However, other members of the family such as the yeast and C. elegans CTLs neither transport choline nor are required for membrane synthesis, since other mechanisms of choline transport exist in these organisms. Even CTL1, which comes the closest to fulfilling the role of a choline transporter dedicated to phosphatidylcholine synthesis, has recently been found in mitochondria as well as the plasma membrane. Together, these findings indicate that both other substrates and other roles than providing choline for phospholipid synthesis need to be examined to better understand the physiological contribution of these proteins.
Question: Do CTL family proteins increase uptake of other amines?
Methods: Recombinant CTL proteins (hCTL1 and hCTL2, CeCTL) were expressed in Xenopus oocytes and uptake of several radioactive

substrates was measured and compared to that of water injected oocytes.
Results: As well as choline, CTL1 and CTL2 also increase uptake of betaine and spermine but not glucosamine. The *C. elegans* ortholog CeCTL/chtl-1 did not transport choline or spermine, but did increase uptake of the amino acid aspartate by cRNA-injected oocytes.
Conclusions: The CTL proteins appear to have a more complex biological role than originally reported. CTL proteins that are known to transport choline have a broader substrate profiles that include spermine, which is known to affect mitochondrial calcium levels and glucose metabolism.

P5 03
A splicing variant leads to complete loss of function of *Betaine-homocysteine methyltransferase* (*BHMT*) gene in hepatocellular carcinoma

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Background: The remethylation of homocyteine into methionine is catalyzed either by methionine synthase (MTR) or by betaine-homocysteine methyltransferase (BHMT), in the liver. Choline/betaine deficiency and impaired BHMT pathway have been associated with hepatocellular carcinogenesis, in animal models. The molecular mechanisms that impair the BHMT pathway are unknown.
Aim: We aimed to investigate *BHMT*, *BHMT2,* and *MTR* expression in HepG2 cells and human hepatocarcinoma tissues.
Methods: Transcripts were quantified by RT-qPCR and splicing was assessed by analysis of exon junctions and sequencing of variants. Protein expression was studied by Western Blot, immunohistochemistry and enzyme activity. Tumor tissue was compared with surrounding healthy tissue.
Results: RT-qPCR of HepG2 cells and of tumor samples showed a strong decrease of transcripts of *BHMT* and *BHMT2*, compared to normal. *MTR* transcript levels were not different. The decreased *BHMT* expression resulted from the transcription of a splicing variant that produced a frameshift in exon 4, with a premature termination codon in exon 5 and a loss of function of the gene. This splicing variant did not fit with any mechanism resulting from known splicing consensus sequences and was not detected in normal adult and fetal liver. BHMT protein and enzyme activity were not detectable in HepG2 and tumor tissues, compared to normal tissues.
Conclusion and Discussion: A transcription variant of exon 4 produces a loss of function of *BHMT* in human hepatocarcinoma. This opens new insights for targeting MTR in chemotherapy of hepatocarcinoma. Whether this abnormal transcription of *BHMT* is part or consequence of liver carcinogenesis should deserve further investigations.

P5 04
Methionine-Homocysteine Metabolism and Methrotexate Toxicity

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Background: Methrotexate (MTX) is a drug widely used in pediatric oncology. Like other chemotherapeutic drugs it can cause severe adverse reactions. It is currently not possible to predict how severe the adverse reactions will be in a particular patient.
Aim: We are conducting a pilot study to identify polymorphisms related to the betaine, homocysteine and methionine metabolism, as well as their metabolites that could predict the occurrence of adverse reactions in patients receiving MTX. This could lead to an improvement of chemotherapeutic protocols, eg with the administration of betaine in patients at high risk of presenting toxicity from MTX.
Methods: We included 5 Patients (3 with ALL, 1 with AT/TR and 1 with medulloblastoma) that were treated with intravenous and intrathecal MTX, according to the COALL, HIT and Eu Rhab chemotherapeutic protocols. During the protocols we collected serial samples of cerebrospinal fluid and blood. We intend to analyze the samples by mass spectrometry and compare the results with MTX serum levels, as well as with the adverse reactions presented by the patients and changes found in the cerebral imaging.
Results: Up to now we have collected 114 blood serum and 79 cerebrospinal fluid samples. Preliminary results show that patients with betaine and homocysteine deficiency o are at increased risk for presenting MTX toxicity.
Discussion and Conclusion: By analyzing polymorphisms and metabolites of betaine, homocysteine and methionine in patients receiving MTX we hope to find a correlation that might improve chemotherapeutic protocols in the future.

P5 05– WITHDRAWN

P5 06
Choline and methyl metabolism in the liver of the laboratory rat fed a high fat diet

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Background: Phosphatidylcholine (PC) is a component of very low density lipoprotein (VLDL) which is critical for lipid secretion from the liver. High fat diets increase VLDL secretion by the liver. PC is synthesised either directly from dietary choline or via the enzyme phosphatidylethanolamine N-methyl transferase (PEMT). The relative activity of these two pathways is unknown in animals fed high fat diets.
Aim: To evaluate the impact of a high fat diet on the expression of genes associated with choline metabolism in the liver of the rat.
Methods: Male Sprague Dawley rats were fed low or high fat diets (Dyets Inc.) for 2 weeks. Livers were removed and levels of key mRNAs were measured by Q-RT-PCR (Applied Biosystems). Data were normalised to the 18S ribosomal RNA.
Results: High fat intake increased liver fat content. The mRNAs for betaine hydroxymethyl transferase (Bhmt), PEMT and PPAR-α were induced by the high fat diet. There was no change in choline dehydrogenase (Chdh), phosphocholine cytidyltransferase (Pcyt), gadd153 or Srebp-1c.
Conclusion: These results suggest high fat diets increase the production of PC via PEMT. Furthermore, the data suggest that the animals are using betaine as methyl source.

Table 1 Gene expression in the liver of rats fed high fat diets **(P5 06)**

		Bhmtr	Chdh	Pcyt	PEMT	gadd153	Srebp-1c	PPAR α
Mean	LF	3.47	3.68	2.59	3.62	3.28	5.23	3.72
	HF	5.29	3.95	2.99	4.60	3.00	4.60	5.41
sd	LF	1.01	0.93	1.51	0.61	0.79	2.05	0.67
	HF	0.65	0.36	1.11	0.46	1.85	1.97	1.37
P		0.003	0.512	0.593	0.008	0.704	0.570	0.010

P5 07
Citicholine ameliorates regresive age-related changes in the mouse hippocampus

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Our goal was to analyze the effects of chronic administration of citicoline (cytidine-5′-diphosphate-choline) on the morphology and features of several hippocampal cell populations such as; granule cells, hiliar neurons, and astrocytes. Three groups (N=60) of male mice (CFW cobs) were used in this study. A group of 12-month-old mice (Adult Control Group -ACG), a group of 24-month-old mice (Old Control Group -OCG), and a third group of mice administered a solution of Citicholine from 12-month of age up to 24 month (Old Experimental Group -OEG). The histological techniques used in this study were; NADPH-diaphorase to evaluate those neurons that synthesize nitric oxide (NO). The features of astrocytes were evaluated using GFAP. Electron microscopy (EM) was used to analyze the morphological features of the hippocampal cells. The cognitive study was performed in a radial maze. The hippocampus of the OCG animals presented some characteristics of ageing when compared to ACG animals. These main features were a significant decrease in the number of NADPH-diaphorase positive neurons in the dentate gyrus (DG) and a significant increase in the number of astrocytes in the OCG animal. When we compared the results of these two groups with those of the OEG, we observed a significant improvement in these ageing effects. The morphological characteristics of neurons and astrocytes were similar to those of the ACG animals. We may conclude that the chronic treatment of these mice with citicoline ameliorates some of the deleterious effects of ageing in some hippocampal cell populations. The citicholine (Somazina) used in these experiments was kindly provided by FERRER-INTERNACIONAL Spain.

P5 08
Hepatoprotective effect of betaine against alcoholic liver injury via improvement of impaired hepatic transsulfuration reactions

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Background and Aims: It has been accepted that alcoholic liver injury involves an impairment of hepatic metabolism of sulfur-containing amino acids. We studied the effects of dietary betaine on the disturbance of transsulfuration reactions in liver injury induced by chronic ethanol intake.
Methods: Rats were fed a liquid diet containing ethanol (5 %) for 6 wk. Betaine (1 %) was supplemented for the entire period or for the final 2 wk of ethanol intake.
Results: Ethanol intake resulted in hepatic lipid accumulation and elevation of serum ALT and AST activities. The resistance of liver tissue to oxidative stress was also reduced significantly. The ethanol-induced changes were all inhibited by betaine supplementation. Ethanol intake reduced hepatic levels of S-adenosylmethionine (SAM), cysteine, glutathione, and taurine. Methionine adenosyltransferase (MAT) and cysteine dioxygenase (CDO) activities were depressed, whereas cystathionine γ-lyase (CγL) was induced. Betaine supplementation increased MAT and CγL, but inhibited CDO further. The ethanol-induced decrease in hepatic SAM and glutathione was reversed by betaine, which may account for the enhancement of antioxidant defense. Increases in hepatic mRNA expression of CD14, TNFα, COX-2, and iNOS were all prevented when supplemented with betaine. A similar degree of hepatoprotection was provided by betaine administered for the final 2 wk of ethanol intake, revealing its potential therapeutic value in alcoholic liver.
Conclusions: Betaine supplementation attenuates the liver injury and inflammatory response induced by chronic ethanol intake. It is suggested that hepatoprotective effect of betaine is associated with improvement of impaired transsulfuration reactions.

P5 09
Associations of folate, vitamin B12, choline, and betaine with homocysteine in a Bangladeshi population

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Background: For reasons that are unclear, Araihazar, Bangladeshis an area with a high prevalence of folate deficiency (FD) and hyperhomocysteinemia (HHcys). Folate and betaine are two interdependent methyl donors used in the remethylation of homocysteine (Hcys) to methionine. Betaine can be obtained from diet or produced from the oxidation of choline.
Aim: To evaluate the relationships of plasma folate, vitamin B12, choline, and betaine with Hcys in this unique population.
Methods: We conducted a cross-sectional study of 379 Bangladeshi adults and measured plasma concentrations of folate, vitamin B12, choline, betaine,SAH, and Hcys.
Results: As previously observed, moderate FD and HHcys were common (FD: males-39%, females-21%; HHcys: males-37%, females-20%). In the total sample, a multiple linear regression model including age, sex, smoking, folate, B12, betaine,SAH, and body mass index accounted for 37.5% of the variability in Hcys (folate: 15.8%, B12: 3.1%, betaine: 1.1%); folate, B12, and betaine were all significantly inversely associated with Hcys. In those with FD (n=111), the same regression model accounted for 42% of the variability in Hcys and the associations of betaine and folate with Hcys were stronger than in the total sample. Among

the folate sufficient (n=268), choline was significantly positively associated with Hcys and the association between betaine and Hcys was not significant.
Discussion and Conclusion: In this Bangladeshi population, nutrients have a strong influence on Hcys concentrations and the associations of choline and betaine with Hcys differ by folate status.

P6 – Folate and Homocysteine

P6 01
Factors associated with vitamin B12, folate and homocysteine levels in persons with type 2 diabetes

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Objective: The purpose of this study was to evaluate the extent of vitamin B12 and folate deficiencies and the levels of homocysteine in type 2 diabetic patients in respect to the metformin exposure and dietary habits.
Research Design and Methods: A cross-sectional study was conducted on 298 type 2 diabetic patients. Fasting serum vitamin B12, folic acid and homocysteine were measured. Demographic characteristics and dietary intake were assessed.
Results: 27.2% of type 2 diabetes had serum vitamin B12 deficiency (<200 pg/ml), 27.2% had folic acid deficiency (<4.43 ng/ml) and 62.8% had serum homocysteine >15 µmol/l. Metformin, serum folic acid, low-fat dairy product, high-fat dairy product and vitamin supplementation were significantly associated with the vitamin B12 deficiency [OR: 1.70 (1.38-2.11); OR: 0.86 (0.78-0.95); OR: 0.53 (0.34-0.81); OR: 2.04 (1.05-3.97); OR: 0.38 (0.17-0.83), respectively] in a logistic regression. Male sex, serum vitamin B12, vitamin supplementations and blood pressure lowering drug were inversely associated with low folic acid levels [OR: 3.77 (1.88-7.57); OR: 0.997 (0.996-0.999); OR: 0.26 (0.13-0.59) and OR: 2.58 (1.31-5.07), respectively]. Hyperhomocysteinemia was significantly associated with male sex and serum vitamin B12 [OR: 2.98 (1.65-5.39) and OR: 0.999 (0.998-1.000), respectively].
Conclusions: The frequency of vitamin B12 deficiency is higher in patients receiving metformin treatment and lower intake of low fat dairy product. The higher serum vitamin B12 is related to the lower serum homocysteine.

P6 02
Hyperhomocysteinemia induces preterm birth in mice via the Gpr109a/Cox-2/PGE2 axis

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Preterm birth, with its increased mortality and morbidity in babies, is the most significant problem in clinical obstetrics. Homocysteine, an intermediate in methionine metabolism, is a risk factor for various placenta-mediated diseases such as preeclampsia, but its role in preterm birth is not known. Here we describe a mouse model of hyperhomocysteinemia (***Cbs*$^{+/-}$** mouse), which is associated with preterm birth with a hundred percent penetrance. Elevated levels of homocysteine found in this mouse upregulate Cox-2 expression and PGE2 production both in uterus and placenta, and consequently initiate premature contractility in the uterine tissue. Administration of a Cox-2 inhibitor abolishes preterm birth in these mice. Gpr109a, a G-protein-coupled receptor for niacin, has pro-inflammatory effects on uterus and induces Cox-2 expression. Homocysteine upregulates GPR109A expression, suppresses the activity of BKCa channel, and depolarizes human myometrial cells. Deletion of ***Gpr109a*** in the hyperhomocysteinemic mice (***Cbs*$^{+/-}$/*Gpr109a*$^{-/-}$**) reverses premature birth. Thus, hyperhomocysteinemia is an etiologic factor in preterm birth, and the process involves the Gpr109a/Cox-2/PGE2 axis. Blockade of this receptor with high-affinity and selective antagonists may have potential as a novel and effective therapeutic approach to prevent premature delivery.

P6 03
Homocysteine, folate and vitamin B12 levels in type 2 diabetic patiens with various stage of chronic kidney disease

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Background: A lowering effect of folate supplementation on homocysteine levels (tHcy) in patients with type 2 diabetes mellitus (T2D) was recently demonstrated by Aghamohammadi V. and collaborators (1), but no data on T2D with chronic kidney disease (CKD) were reported.
Aim: Aim of the study is to determine tHcy levels in T2D patients at various stages of CKD, and the relationship with folate and vitamin B_{12}.
Methods: We recruited 40 T2D-CKD patients (stages II-IV according to K-DOKI guidelines). tHcy levels were measured as previous decribed (2). Folate and vitamin B_{12} were assayed by an automated chemiluminescence system (Advia Centaur; Siemens).
Results: tHcy levels increases in all patients, and this rise positively correlate with the CKD stage ($p<0.05$). Moreover, we interestingly found that only in stage 2 CKD tHcy concentrations negatively correlates with low levels of both vitamin B12 and folate, whereas in stage 3 folate have no effect, and in stage 4 vitamin B_{12} have no effect on tHcy concentrations.
Conclusion: We demonstrated for the first time that tHcy increase in diabetic CKD patients and this rise correlate positively with the stage of kidney disease. Folate and vitamin B_{12} levels have different effect on tHcy levels at different stages of CKD. These results may indicate the importance of the combined folate and vitamin B_{12} therapy at early stage of CKD, where the levels of tHcy negatively correlates with both folate and vitamin B_{12}.

P6 04
Effectiveness of short-term low-dose folic acid supplementation in Polish elderly

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Background: Serum homocysteine (Hcy) concentration depends on nutritional factors, including B-vitamin intake, but genetic influencesalso contribute to this trait. The significant effect of the *MTHFR* C677T polymorphism has been demonstrated.
Aim: The aim of the present study is to evaluate the impact of folic acid supplementation on serum homocysteine levels and blood biochemical parameters among Polish elderly people with differing C677T *MTHFR* genotypes.
Methods: 151 men and women over 60 years of age (69.8 ± 7.7) were recruited from the University of the Third Age and the Social Welfare Home in Poznań. All participants were supplemented with 0.4 mg/d folic acid for 8 weeks. The serum folate level was determined immunoenzymatically, and plasma Hcy was measured using the HPLC method. Blood biomarkers were analyzed using a Vitalab Flexor biochemical analyzer. *MTHFR* genotyping was performed using the PCR-RFLP method.
Results: Basal serum Hcy concentration ranged from 4.0 to 18.7 µM, while after folic acid supplementationa reduction in concentration was observed ($p<0.001$). The size of the decrease in homocysteine level was dependent on the *MTHFR* genotype, being 1.4 µM in people with the CT or TT genotype, but only 0.7 µM in CC homozygotes. Hcy concentration after folic acid supplementation was correlated with body weight and waist-to-hip ratio. Moreover, after dietary intervention the T-allele carriers had decreased HDL cholesterol and increased glucose concentration.
Conclusion: Short-term low-dose folic acid supplementation does reduce serum homocysteine concentration in the elderly, but is more effective in people with the CT or TT *MTHFR* genotype. However, such dietary intervention can change also lipid metabolism.

P6 05
Effect of Folic Acid Supplementation on Homocysteine, Serum Total Antioxidant Capacity and Malondialdehyde in Patients with Type 2 Diabetes Mellitus

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Objective: Metformin is widely used in patients with type 2 diabetes but may decrease vitamin B12 and folate levels and increase levels of homocysteine. Hyperhomocysteinemia and hyperglycemia induce oxidative stress in type 2 diabetes. Thus, this study performed to determine the effects of supplementation of folate on concentration of homocysteine, total antioxidant capacity, malondialdehyde.
Methods: This was a double-blind randomized controlled clinical trial. 68 men with type 2 diabetes participated voluntarily with written consents. Patients were randomly divided in two groups; folic acid 5mg/day and placebo. All the patients received the tablets for 8 weeks. Anthropometric and nutrient intakes data obtained from each patient. Baseline and 8th week homocysteine, total antioxidant capacity, malondialdehyde, folate and B12 levels were measured.
Results: After folate supplementation in folic acid group, homocysteine was significantly decreased (15.13±3.25 to 12.1±3.15µmol/L, P0/05).
Conclusions: Pharmacological dose of folate supplementation effectively lowered plasma homocysteine and serum malondialdehyde levels and improved the total antioxidant capacity in patients with type 2 diabetes.
Key words: Homocysteine, Malondialdehyde, Type II Diabetes Mellitus, Total Antioxidant Capacity.

P6 06
C677T Polymorphism of the Methylenetetrahydrofolate Reductase Gene and Hyperlipidemia affect Folic Acid, and Vitamin B12, but not Homocysteine Serum Levels in Algerian Subjects with Cardiovascular Disease

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Methods: This research was carried out as a prospective study on 98 patients hospitalized in the Cardiology Section. Mean age of participants was 57 y (range 20-96 y). The genetic analysis of the MTHFR C677T polymorphism was performed by real-time polymerase chain reaction on a Light Cycler. The concentration of tHcy, folic acid and vitamin B12 levels were determined using a competitive immunoassay method. Plasmatic total cholesterol, triglyceride, glucose, creatinine and urea concentrations were measured by the colorimetric method.
Results: Plasma tHcy was significantly higher in the patients with CVD, this HHcy was associated with the presence of mildly elevated serum urea and creatinine ($p < 0.05$). MTHFR gene mutation does not seem to be associated with elevation of plasma tHcy in the studied patients and this lack of correlation could be explained by the level of folatemia which seem must to be more lower than in our study. CVD patients with 677CT/TT genotypes had a higher concentration of total cholesterol (TC) than those with 677CC genotype ($p < 0.05$). Although, the presence of 677T variant conjugated with hypofolatemia (<15.4 ng/mL) had a more detrimental effect on the level of TC ($p < 0.05$). Folatemia and vitamin B12 were much higher in 677CC genotype compared to 677CT/TT genotype in CVD subjects without hyperlipidemia ($p < 0.05$), however in patients with hyperlipidemia these values became lower also with 677CC genotype.
Conclusions: Hyperlipidemia affects the levels of plasmatic folate and vitamin B12 indepedently to mutated MTHFR genotype. The effect of 677T variant on TC, folate and vitamin B12 can be explained by the adverse effect of elevated tHcy on lipid profiles and on plasmatic folate and vitamin B12.

P6 07
Decreased activity of folate transporters in lipid rafts resulted in reduced folate uptake in chronic alcoholism in rats

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Background: Folic acid is an essential nutrient, required for one-carbon biosynthetic processes and methylation of biomolecules.

Deficiency of this micronutrient leads to disturbances in normal physiology of cell. Chronic alcoholism is well known to be associated with folate deficiency which is due, in part to folate malabsorption.
Aim: The aim of present study was to delineate the regulatory mechanisms of folate uptake in liver in an in vivo model of chronic alcoholism.
Methods: Male Wistar rats were fed 1 g/kg body weight/day ethanol (20% solution) orally for 3 months and the kinetics of folate uptake and expression of transporters were studied in liver basolateral membrane vesicles.
Results: The folate transport system in liver basolateral membrane (BLM) was found to be carrier mediated with pH optima at acidic pH, with the major involvement of proton coupled folate transporter (PCFT) in the uptake. The folate transporters were found to be associated with lipid raft microdomain of liver BLM. Moreover, ethanol ingestion decreased the folate transport by altering the Vmax of folate transport process and downregulating the expression of folate transporters in lipid rafts. The decreased transporter levels were associated with reduced protein and mRNA levels of these transporters in liver and consequently resulted in reduced folate levels in liver of ethanol fed rats.
Discussion and Conclusion: The chronic ethanol ingestion led to decreased folate uptake in liver, which was associated with the decreased number of transporter molecules in the lipid rafts at the liver BLM, which can be ascribed to the reduced synthesis of these transporters.

P6 08
Paradoxical role of H475Y Glutamate Carboxypeptidase II (GCPII) genetic polymorphism in altering disease susceptibility

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Glutamate Carboxypeptidase II (*GCPII*) inhibition was found to confer protection against certain neurological disorders and cancer. Despite the pivotal role of this enzyme, the most common polymorphism in this crucial gene i.e.; H475Y has not been explored comprehensively in all its splice variants. In the current study, we have determined the role of this variant in different disease conditions such as breast and prostate cancers, autism, coronary artery disease (CAD) and miscarriages (N=1561). The analysis of this variant was done using PCR-RFLP, which was further confirmed by dideoxy sequencing. Axysm folate kit was used to measure the plasma folate levels. *GCPII* expression was studied by semi quantitative RT-PCR. *In silico* model was developed using PYMOL. We observed protective role of H475Y variant in cancers [breast cancer; OR (95% CI): 0.81 (0.55-1.19), prostate cancer: 0.00 (0.00-0.66)], and in autism (OR 0.47 (0.21-1.03), on the other hand inflated risk was observed in CAD (OR 1.69 (1.20-2.37) and miscarriages [Maternal OR 3.26 (2.11-5.04); Paternal 1.99 (1.23-3.21)]. Further, this variant was found to impair the intestinal folate absorption in subjects with dietary folate intake in the lowest tertile (CC vs. CT in lowest tertile; 7.56±0.85ng/ml vs. 2.73±045ng/ml, p=0.005). *In silico* model of GCPII showed steric hindrance with H475Y resulting in stereochemical alteration of catalytic site, thus interfering with ligand binding. There was no statistically significant association was observed between the dietary folate levels and *GCPII* expression. However, a positive correlation was seen between the plasma folate levels and *GCPII* expression (r=0.70, p<0.05). To conclude, our data suggests the inhibitory role of H475Y variant on folyl glutamate carboxypeptidase and N-acetylated α-linked acidic dipeptidase activities and probable inhibition of prostate specific membrane antigen.

P6 09
Hyperhomocysteinemia and markers of inflammation and oxidative stress in patients with unstable angina pectoris

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A comparison of levels of homocysteine (HC) and markers of oxidative stress (OS) and inflammation in the systemic blood flow is a topical problem in cardiology, taking into consideration a multifactor character of processes that lead to destabilization of the atherosclerotic plague.
Nineteen patients with acute coronary syndrome and unstable angina (group 1, aged 57±1 years) as well as 17 patients with coronary heart disease and stable angina (group 2, aged 56±2years) were included in this study. Plasma concentrations of HC were determined by high performance chromatography [Williams R.H. et al., 2001]. Serum concentrations of high sensitivity C-reactive protein (hs-CRP) were measured by ELISA method. The concentrations of thiobarbituric acid reactive substances (TBARS) in plasma [E.N. Korobeinikova, 1989] and the activity of blood superoxide dismutase [Chevari S. et al., 1991] were determined spectrophotometrically. Spontaneous platelet aggregation and ADP-induced platelet aggregation in platelet-rich plasma were assessed by aggregometry AP-2110 (SOLAR, Minsk, Belarus).
It was shown that in patients with unstable angina, hyperhomocysteinemia amounted to 21.4±3.7 and in those with stable angina - 12.8±1.4 (p=0.05). The variations in hs-CRP level were more pronounced: 8.82±2.54 and 2.05±0.30 mg/l, respectively (p=0.001. Under unstable angina, platelet spontaneous and ADP-induced aggregation and TBARS were tended to increase, whereas superoxide dismutase activity was elevated by 80% (p=0.05). Correlation between the rates of coronary artery lesions and hsCRP concentrations was not found.

P6 10
Folates induce human erythroleukemic cell line proliferation through foate receptors α1 and Notch1 signaling

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Introduction: Folic acid (FA) consumption at high levels has been associated with cancer risk. Several mechanisms have been proposed to explain this association. The Notch signal pathway and folate receptors (FR) have been implicated in the regulation of cellular proliferation.
Aim: To demonstrate that high concentrations of FA or its reduced form, 5-methyltetrahydrofolic acid (5-MTHF) increase human

erythroleukemic cell line K562 cell proliferation through an alteration of Notch1 and/or folate receptors α1 (FR α1) expression.
Methods: K562 cells were cultured in high (100nM), low (10nM) or 0nM FA or 5-MTHF concentrations during 72 hours. Cell proliferation was determined by the MTT method, Notch1- intracellular domain (NICD) as well as Folate receptor FR α1α1 (FR α1) levels were analyzed by flow cytometry.
Results: K562 cells exposed to 100nM FA or 5-MTHF showed higher proliferation rate than those exposed to 10 nM of FA or 5-MTHF ($p<0.01$) during 72h. On the other hand, Notch-1 intracellular domain expression was reduced at higher FA or 5-MTHF concentrations compared with lower concentrations (0-10nM) ($p<0.01$). Total FR α1 expression was similar at both FA or 5-MTHF concentrations (10 or 100 nM), however, external FR α1 expression was lower at higher FA or 5-MTHF concentrations ($p<0.05$).
Conclusion: These data suggest that FA and 5-MTHF at high concentrations may induce down regulation of FR α1 inhibiting Notch1 signaling conducing to K562 cells proliferation.

P6 11
Increased level of serum Homocysteine in vitiligo

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Background: Vitiligo is an acquired depigmenting disorder caused by the destruction of melanocytes. The exact aetiopathogenesis and mechanisms of vitiligo are not fully understood.
Vitamin B12 and folic acid levels are decreased in vitiligo, which are the important cofactors required in the metabolism of Homocysteine (Hcy). Consequently, Hcy level increase in the circulation. Therefore it is possible that increased Hcy plays a role in melanocytes destruction. The aim of this study is to look for any association of vitiligo with serum Hcy level.
Method: Total 30 patients of both sexes with vitiligo and 30 control subjects were enrolled in this study. Sera from patients and controls were assayed for Hcy by Enzyme immunoassay. The collected data were analysed by SPSS version-16.
Results: The mean serum level of Hcy was significantly higher in patients with vitiligo as compared to healthy controls and its level was high in male patients as compared to female patients. The Hcy level in vegetarian patients was significantly higher as compared with non-vegetarian patients. The Hcy level was also significantly higher in active vitiligo patients as compared to stable vitiligo patients.
Conclusion: An increased serum Hcy may be a precipitating factor for vitiligo in the predisposed individuals. Serum Hcy is related to gender of patients, activity of disease and dietary habits of vitiligo patients.
Keywords: Vitiligo, homocysteine, melanocytes, dietary habits.

P6 12
Metabolically dependence of different dietary folic acid levels on methionine metabolism in growing rats under induced vitamin B12 deficiency

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In previous studies, we have demonstrated that dietary FA deficiency compromises methionine metabolism, whereas supplementation does not show additional positive effects compared to control diet in growing animals. However, there is not information about the FA supplementation/deficiency effect on the methionine cycle under induced dietary vitamin B_{12} in deficiency weaning rats.
The present study was conducted to examine the effects on the methionine/methylation metabolism during growth according to different dietary levels FA and vitamin B_{12}.
Male Sprague Dawley rats (6 weeks) were fed diets containing 0 or 50 µg vitamin B_{12}/kg diet with 0, 2 or 8 mg FA/kg diet for 30 days.
Serum folate was higher in rats fed a FA control and supplement diet when there was B_{12} deficiency compared to corresponding B_{12} control dietary levels. These results suggested that vitamin B_{12} deficiency originates a cellular folate accumulation in the methyl form, creating a methylfolate trapping scenario. For the three different FA treatments plasma homocysteine level, hepatic S-adenosylmethionine (SAM), hepatic and cerebral S-adenosylhomocysteine (SAH) concentrations were also higher in B_{12} deficient groups. However, hepatic DNA methylation was lower in the three groups under B12 dietary deficiency. This indicates that both vitamins are necessary for a normal and correct functioning of the cycle during growth and to avoid possible risks associated to alterations in these metabolic pathways, as we have demonstrated during pregnancy in other studies. In conclusion, metabolic priories in relative to the methionine metabolism during growing are clearly associated to an adequate folic acid/B12 ratio. More studies are being/carry on in order to achieve optimal intakes of the FA/B12 ratio for different situations (e.g. high methylation demand periods).

P6 13
The level of homocysteine and apoptotic cells in epilepsy patients treated with antiepileptic drugs

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Antiepileptic drug (AED) therapy of patients with epilepsy may generate a lot of plasma molecular changes including the concentration of homocysteine (Hcy). Some of the research showed that Hcy may induce apoptosis.
The aim of the study was to analyze the level of plasma Hcy and its influence on expression of the apoptotic proteins (p53, Bax, Bcl-2) in peripheral lymphocytes and the level of apoptotic cells in epileptic patients, before and during AED treatment {VPA, carbamazepine (CBZ) and lamotrigine (LTG), in monotherapy and polytherapy}, and in controls.
There were 23 epileptic patients at the age of 18 to 69 in the study group, 20 of them were treated with AEDs, 3 before initiating the convulsive treatment. Control group consisted of 22 individuals at the age of 22 to 61. The Hcy level was analyzed by HPLC, the levels of apoptotic proteins in peripheral lymphocytes by western blotting method and the level of lymphocyte apoptotic cells by active caspase -3 detection by flow cytometry.
The studies revealed the elevated level of Hcy (> 16 µm) in 65% of epileptic patients treated with AEDs, especially with VPA. Mild hyperhomocysteinemia (HHcy) was combined with lower expression of p53 protein but higher ratio Bax/Bcl-2 and increased level of apoptotic cells.

It appears that some AEDs generate HHcy which may stimulate apoptotic processes. The mechanism of apoptosis induction by AEDs needs further studies.

P6 14
The levels of vitamins and aminothiols involved in homocysteine metabolism in the blood plasma of childbearing age women.

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The emergence of hyperhomocysteinemia (HHCy) as a marker and pathogenetic factor in various diseases is closely related to the metabolic, genetic and nutritional factors. Investigation of the extended spectrum of biomarkers involved in the HHCy pathogenesis is especially important for perinatal pathology.
In 140 young women aged 17 to 39 years (25.4 ± 5.4 years) voluntarily participated in the study, blood plasma folate (FA) levels were examined by radioassay method; cobalamin (B_{12}) - by chemiluminescence microparticle intrinsic factor assay; homocysteine (HCy), cysteine (Cys) and cystenile-glycine (Cys-Gly) - by HPLC method.
It was found that the levels of vitamins and aminothiols are: FA - 13.0 [8.0;23.5] nmol/l; B_{12} - 256.0 [228.0;339.0] pmol/l; HCy - 7.53 [6.7;9.2] umol/l; Cys - 190.5 [176.7;202.2] umol/l; Cys-Gly - 30.6 [26.9;35.2] umol/l. According to percentile analysis 5% of women have unsatisfactory FA and B_{12} status, which is consistent with the development of moderate HHCy. The level of total Hcy in blood plasma has a moderate relationship with the content of the FA ($r= -0.39$, $p<0.01$) and weakly significant trend with the content of vitamin B_{12} ($r= -0.21$, $p<0.05$). The strongest correlation was found for the levels of HCy and Cys ($r=0.48$, $p<0.01$).

P6 15
N-Homocysteinylation causes hair keratin damage in humans and animals

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Background: Genetic or nutritional disorders in homocysteine (Hcy) metabolism elevate Hcy-thiolactone (HTL) are associated with heart and brain diseases. Hcy becomes a component of protein as a result of *N*-homocysteinylation of protein lysine residues by HTL. This reaction causes a thyil radical-mediated protein damage, which would accumulate in long-lived proteins and could contribute to Hcy-related pathology.
Aim: To gain insights into a role of *N*-homocys-teinylation in inducing protein damage we studied Hcy content of hair keratin.
Methods: N-linked Hcy after conversion to HTL was analyzed by HPLC method with fluorescent detection.
Results: *N*-linked Hcy comprised 33% of all Hcy content in human hair keratin and the remaining 67% was *S*-linked. Hair keratin from wild type C57BL/6J mice fed with a high methionine diet contained 2.2-fold more *N*-linked Hcy than control mice. *N*-linked Hcy was elevated 20-, 7.3-, or 2.5-fold in hair keratin from *Cbs-/-*, *Cse-/-*, or *Mthfr-/-* mice, respectively. SDS-soluble *N*-linked Hcy comprised a 10-fold smaller fraction of SDS-soluble + SDS-insoluble *N*-linked Hcy in hair keratin from hyperhomocysteinemic mice than from wild type animals (0.04±0.00 *vs*. 0.39±0.04, $p=0.0003$).
Discussion and Conclusion: *N*-linked Hcy is a component of human and animal hair keratin. Our findings demonstrate that hair keratin is a target for *N*-homocysteinylation *in vivo* and that the modification causes severe damage to hair keratin, manifested by the loss of SDS-solubility.

P6 16
Elevated Homocysteine Level is a potential Risk factor for osteoporosis among elderly population of Nepal

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Background: Low B-vitamin status and elevated homocysteine is a strong risk factor for osteoporosis among elderly population of Nepal.
Objective: Our objective was to examine the associations of plasma concentrations of vitamin B 12 and homocysteine with osteoporotic risk in elderly men and women.
Material and Methods: This was a hospital based observational study conducted in the patients visiting the department of biochemistry, Manipal teaching hospital of Nepal. A total of 1500 men and women (mean age 78 yrs) was incorporated in the study. Blood samples were collected from 2010 to 2011 were used to categorize participants plasma B-vitamin (normal, low, deficient) and homocysteine (normal, high) groups. Descriptive statistics and testing of hypothesis were used to analyse the data with SPSS 16.
Result: Vitamin B 12 was inversely associated with osteoporotic risk ($p<0.05$), yet associations were somewhat attenuated and not significant after controlling for BMD, and homocysteine. Participants with high homocysteine (>14µmol/liter) had approximately 80% higher osteoporotic risk but this association was attenuated after controlling for vitamin B12 (hazard ratio=1.80; 95% confidence interval 1.22, 2.77).
Conclusion: Low B-vitamin concentration may be a risk factor for decreased bone health, yet does not fully explain the relation between elevated homocysteine and osteoporotic.

P6 17
A link between systems: functional integration of the age-related disorders including folic acid, vitamin B12 deficiency and hyperhomocysteinemia

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Background: Folic acid/vitamin B12 deficiency and elevated serum homocystein levels (hyperhomocysteinemia) have been recognised as mutually related disorders, all of them associated with many ageing phenotypes.

Aim: To achieve better understanding of the relationships among these important age-related disorders, we reached out for a systems biology methodology approach.
Methods: The sample,consisted of 93 patients aged 50-89 years (median 69), characterised with multiple chronic health disorders, were divided into two groups, according to the homocystein cut-off value indicating hyperhomocysteinemia (>12mM). Their health-status were determined systematically, by using a large amount of parameters, including both clinical and laboratory ones. Data mining method was used to find patterns in the data. By subsequent data mining, applied on particular of selected parameters, it was possible to get closer insight into the way selected parameters are interconnected forming a functional network.
Results: Constructed network linked, with one another, parameters indicating: folic acid and vitamin B12 deficiency, hyperhomocysteinemia, low creatinine clearance, the thyreoid gland hypofunction, and neuropsychiatric and gastroduodenal disorders, as well.
Discussion and conclusion: By using this method, many hidden relationships within the biological network including folic acid/vitamin B12 deficiency and hyperhomocysteinemia, have become visible, thereby improving our understanding of the issue. In a more broader context, intervening inside the network, might be the therapeutic strategy for the future.

P6 18
Hyperhomocysteinemia is associated with venous thromboembolism

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Background: Although hyperhomocysteinemia is an established cardiovascular risk factor, the role in venous thromboembolism (VTE) is still controversial. According to some studies, mild or moderate levels of hyperhomocysteinemia are associated with 2.5-2.95 increased risk for VTE; the administration of folate and vitamins B reduces the recurrences of thrombotic events with 16%, whereas other studies failed to prove the benefit.
Aim and Method: We aimed to detect the association between increased levels of homocysteine and VTE. We performed a transversal, case-control study, including 127 patients diagnosed with VTE and 100 sex- and age matched controls, without VTE. Patients with chronic renal failure, thyroid diseases, or following therapy with methotrexate or vitamins B were excluded, due to the influence of homocysteine levels. Homocysteinemia was assessed using liquid chromatography high pressure reverse phase.
Results: The mean value of homocysteinemia was 17.39 micromol/L. in VTE group and 14.15 micromol/L.in controls, p=0.025. Patients with deep venous thrombosis (DVT) and hyperhomocysteinemia did not present a significantly higher risk for pulmonary embolism in comparison with controls, p=0.127. Hyperhomocysteinemia was significantly associated with VTE, even when controlling the variables age, smoking and cancer, which represent risk factors for DVT as well as factors increasing homocysteinemia, p=0.127.
Conclusions: hyperhomocysteinemia is significantly associated with VTE and represents an independent risk factor for VTE.
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P6 19
Antiepileptic Drugs and Bone Disease: Multifactorial Mechanisms and Role of Homocysteine

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Bony adverse effects are amongst the potentially adverse clinical consequences with antiepileptic drugs (AEDs). Various pathophysiological mechanisms of AED-induced bone disease have been proposed, the most important being the hepatic induction of cytochrome P450 leading to increased metabolism of vitamin D. Other mechanisms include secondary hyperparathyroidism, calcitonin or vitamin K deficiency, deprived estrogen levels, aromatase inhibition etc. Antiepileptic drugs also interfere with circulating homocysteine (hcy) and enhance the susceptibility of epileptics to hcy-related adverse effects. A better understanding of these mechanisms can aid clinicians in identifying and monitoring vulnerable patients and in defining the optimal therapy for all affected patients.
For the management of AEDs associated concomitant bony effects, anti-osteoporotic agents can be prescribed. In our lab, we have focused on developing a model to study AED-induced changes in the bone and the effect of bisphosphonates in alleviating the AED-induced bone loss in rodents. We evaluated the effect of bisphosphonates on antiepileptic efficacy of phenytoin and AED-induced alterations in the biochemical markers of bone turnover, in comparison with calcium and vitamin D_3 (CVD) supplementation. Role of serum hcy and folic acid was assessed. We found bisphosphonates to lower serum hcy due to its antioxidant profile and not via B-vitamin dependent remethylation pathway of hcy. With the newer AEDs gaining importance, our future experiments would focus on comparing them with the conventional AEDs and to examine their impact on multiple aspects of bone health and to identify anti-osteoporotic agents that can be prescribed safely with other AEDs.

P6 20
Correlation of Homocysteine with Vitamins B_{12} and Folate in Indian Patients of Vascular Disease

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Background: The burden of vascular disease has shifted to the developing countries. Hyperhomocysteinemia, which can be caused by genetic polymorphisms as well as dietary deficiencies, has been seen to be more commonly associated with vascular disease in the Indian population as compared to the western populations.
Aim: To identify the correlation between homocysteine levels and B_{12} and folate levels as well as MTHFR C677T polymorphism in Indian patients of vascular disease.
Method: Blood samples of 70 patients of vascular disease and 70 controls were subject to estimation of homocysteine, vitamin B_{12}, folate and the presence of MTHFR C677T polymorphism (heterozygous and homozygous). The data was analysed by the SPSS version 17 statistical package.
Results: Homocysteine levels were observed to be significantly higher ($p<0.001$) and folate levels were significantly lower ($p<0.001$) in Indian patients of vascular disease as compared to those in the controls. Correlation studies revealed that in the controls, homocysteine levels bore a significant($p=0.001$) negative correlation with vitamin B_{12} levels, whereas in the patients, there was a significant negative correlation with B_{12}($p<0.001$) as well as folate levels($p<0.001$).

Homocysteine levels did not bear a significant correlation with the presence of MTHFR C677T polymorphism.
Discussion and conclusion: Since, hyperhomo-cysteinemia is associated with vascular disease in Indian patients, large-scale means to lower homocysteine should be instituted to reduce the prevalence of vascular disease. One such means could be the fortification of food with folate as well as vitamin B_{12}.

P6 21 presented as oral presentation

P6 22
Hyperhomocysteinemia and cognitive dysfunction in Parkinson's disease

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Background: Idiopathic Parkinson's disease (PD) is the most common age-related, neurodegenerative disease of unknown etiology. A part of PD patients have cognitive dysfunction in their clinical course. Several studies demonstrated that hyperhomocysteinemia, which in PD resulted partly form L-Dopa administration, would be a risk factor for cognitive dysfunction.
Aim: The aim of this study was to investigate the relationship between homocysteine (Hcy) and cognitive dysfunction in PD and Alzheimer disease (AD).
Methods: Plasma total Hcy and serum folate, vitamin B12 levels were studied 51 PD without cognitive dysfunction (PD-), 56 PD with cognitive dysfunction (PD+), 26 AD, and 145 non-demented aged controls (CTL). Cognitive dysfunctions estimated by MMSE, WAIS-R, and SDS in these groups were considered. We conducted multivariate analysis: $p<0.05$ was considered to be significant.
Results: Mean Hcy level in CTL was 8.3 nmol/ml and the significantly lowest among the groups. Mean Hcy level in PD+ was 15.0 nmol/ml and significantly higher than those in CTL, PD- (11.7 nmol/ml) and AD (12.1 nmol/ml). Hcy levels were positively correlated with ages and negatively correlated with serum folate concentration. In PD+, Hcy levels were negatively correlated with MMSE, IQ scores in WAIS-R, and SDS scores.
Discussion and Conclusion: The increase of Hcy in PD was seemed to be related to cognitive dysfunction. Hcy is suggested to be a predictive marker for cognitive dysfunction in Parkinson's disease.

P6 23
Dimethylarginines, homocysteine metabolism and cerebrospinal fluid markers for Alzheimer's disease

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Background: Dimethylarginine and homocysteine metabolism are closely linked and alterations of both have been observed in plasma and cerebrospinal fluid (CSF) of patients with Alzheimer's disease (AD). CSF parameters of homocysteine metabolism have recently found to be associated with the AD CSF biomarker phosphorylated tau (ptau) in AD patients.
Objective: To investigate possible relationships between homocysteine and dimethylarginine metabolism and the AD CSF biomarkers ptau181 and amyloid beta 1-42 (Aβ42).
Methods: We assessed parameters of homocysteine (CSF homocysteine, SAH, SAM, 5-MTHF) and dimethylarginine metabolism (plasma and CSF asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine) as well as CSF Aβ42 and ptau181 in 98 controls and 51 AD patients. Logistic regression analyses were performed to assess interrelations between each parameter.
Results: SAH concentrations independently predicted CSF ADMA levels, and CSF ADMA and L-arginine predicted ptau181, but not Aβ42 concentrations in AD patients. When including concentrations of Hcys, 5-MTHF, SAM and SAH into the analysis, CSF ADMA concentrations independently predicted ptau181 levels in AD patients but homocysteine-related metabolites were associated with ptau181 only when ADMA was removed from the analysis model.
Conclusion: These results suggest that CSF ADMA may interact with CNS homocysteine metabolism and may contribute to neurodegeneration and accumulation of phosphorylated tau in AD. Functional and interventional studies are needed to further proof this hypothesis.

P6 24
Homocysteine - induced seizures: aggravation by iNOS inhibition

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Homocysteine and its metabolites, common risks factor for many cardiovascular and CNS disorders, induces seizures via different, but still not-well-known mechanisms. We showed recently the involvement of gasotransmitter NO, produced by family of NO synthases (NOS), in homocysteine convulsive activity, but the role of inducible NOS (iNOS) has not been studied.
The aim of the present study was to examine the effects of aminoguanidine, selective inhibitor of iNOS, on seizures in adult rats induced by subconvulsive dose of D,L homocysteine-thiolactone (Hct).
Adult male Wistar albino rats were intraperitoneally (i.p.) treated with Hct 5.5 mmol/kg and observed for seizure behavioral manifestations during next 90 min. Increasing doses of aminoguanidine (50, 75 and 100 mg/kg, i.p., n=8 per group) or saline (n=9) were injected 30 min prior to Hct administration. Seizure behavior was assessed by seizure incidence, latency time to firs seizure onset, number of seizure episodes and its severity assessed by descriptive scale with 4 grades.
Seizure incidence was significantly increased by aminoguanidine 75 ($p<0.05$) and 100 mg/kg ($p<0.01$). Aminoguanidine in dose-dependent manner also significantly increased number of seizure episodes induced by Hct and its severity. Seizure latency time was significantly shortened in rats receiving aminoguanidine 100 mg/kg ($p<0.05$) before subconvulsive dose of Hct.

It could be concluded that aminoguanidine, iNOS inhibitor, markedly aggravates behavioral manifestations of Hct-induced seizures in adult rats, showing functional involvement of iNOS in homocysteine convulsive mechanisms.

P6 25
Prevalence of Homocysteine in patients with cardiovascular disorders in Pakistan

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Cardiovascular disease (CVD) describes conditions caused by an interrupted or diminished blood flow through the coronary arteries to the heart muscle. The main CVD are heart attack, angina, stroke and peripheral vascular disease.CVD is determined by many risk factors such as age, gender, diabetes, Dyslipidemia, high blood pressure, smoking and elevated levels of risk markers lipoprotein a, Homocysteine (tHcy), C-reactive protein and uric acid. Elevated tHcy has also been established as an independent risk factor for CVD in type 2 diabetic patients compared to non-diabetic patients. In Pakistan over the past three decades, the incidence of coronary heart disease has increased. A study was conducted to investigate the prevalence of plasma tHcy in CV patients, males and females, belonging to two age groups. i.e. below and above 50 years. Homocysteine was determined in plasma quantitatively with IMMULITE 1000 systems. The control value for tHcy in females was observed as 9.15±2.02 in the age group less than 50 years and 9.97±1.68 in the age group above 50 years. Males showed a higher value of 10.5±1.98 in the age group less than 50 years and 9.9±3.2 in the age group above 50 years. Reference values for adult male and female have been reported as 5-15mmol/l respectively.It was further reported that female in reproductive age have lower tHcy values than males and postmenopausal women. tHcy is responsible for various atherosclerotic events. It is reported that Homocysteine concentration in serum/plasma of healthy individual varies with age, gender, geographical distribution and genetic factors. Overall, male showed higher concentration of plasma Homocysteine when compared with their female counterpart.

P7 – Genetics & Epigenetics

P7 01
Cystathionine β-synthase deficiency causes infertility by impairing decidualization and gene expression networks in uterus implantation sites

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Hyperhomocysteinemia has been reported in human reproduction as a risk factor for early pregnancy loss, preeclampsia and congenital birth defects like spina bifida. Female infertility was also observed in cystathionine beta synthase deficient mice (*Cbs*-KO) as an animal model for severe hyperhomocysteinemia. The aim for the present research was to elucidate the time-point of pregnancy loss and to pinpoint gene and cellular changes involved in the underlying pathological mechanism. By mating 90-day-old wild-type and *Cbs*-KO female mice with their homologous male partners, we found that pregnancy loss in *Cbs*-KO occurred between the 8th and 12th gestation day during placenta formation. DNA micro-arrays were carried out on uterus from implantation and inter-implantation samples obtained on day 8th. The results allowed us to select changing genes potentially involved in embryo death which were individually confirmed by RT-qPCR and their expressions were also followed throughout pregnancy. We found that changes in expression of *Calb1, Ttr, Expi, Inmt, Spink3, Rpgrip1, Krt15, Mt-4*, *Gzmc, Tdo2* and *Afp* were important for pregnancy success, since a different regulation in *Cbs*-KO mice was found. Also, differences in relationships among selected genes were observed, indicating a dysregulation of these genes in *Cbs*-KO females. In conclusion, our data provide more information on the gene expression cascade and its timely regulated process required for a successful pregnancy. In addition, it unveils new potential avenues to explore further investigations in pregnancy loss.

P7 02
Relationship between plasma B-vitamins and LINE-1 methylation in leukocytes of patients with a history of colorectal adenomas

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Background: Considerable epidemiological evidence suggests that high intake of certain B-vitamins is associated with a decreased risk of colorectal cancer.Folate and other B-vitamins play a key role in DNA methylation. Global DNA methylation is thought to contribute to carcinogenesis by affecting e.g. chromosomal stability and gene expression regulation.
Aim: The aim was to evaluate whether methylation of LINE-1 repetitive elements is related to plasma concentrations of B-vitamins among patients with at least one histologically confirmed colorectal adenoma ever in their life. We also investigated if these relationships were modified by family history in a first degree relative.
Methods: Global DNA methylation levels in leukocytes of 281 colorectal adenoma patients were estimated using LINE-1 bisulfite pyrosequencing and were expressed as %5-methylated cytosines over the sum of methylated and unmethylated cytosines. Multivariable linear regression was used to analyze the correlation between B-vitamins and LINE-1 methylation.
Results: Preliminary results revealed significant inverse associations between plasma folate, plasma pyridoxal (PL), and plasma 4-pyridoxic acid (PA) with LINE-1 methylation in those with at least 2

lifetime adenomas. Additional analyses revealed significant inverse associations between plasma folate (β=-3.54, 95%CI=-6.92, -0.16), plasma riboflavin (β=-2.51, 95%CI=-4.71, -0.31), plasma pyridoxal 5'-phosphate (β=-4.51, 95%CI=-8.44, -0.57), and plasma PA (β=-2.92, 95%CI=-5.62, -0.21) with LINE-1 methylation in those with at least 2 adenomas and family history of CRC.
Discussion and Conclusion: Our results show statistically significant inverse associations between B-vitamins and LINE-1 methylation in those with at least two lifetime adenoma. Family history of colorectal cancer modified these associations.

P7 03
FB1 fumonisin treatment acts synergistically with methyl donor deficiency during rat pregnancy to produce alterations of H3- and H4- histone methylation patterns in foetuses

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Background: Folate derivatives transfer one-carbon units inside the cell. Prenatal methyl donor malnutrition leads to epigenetic alterations that could enhance susceptibility to disease in later life. Methyl donor deficient diet induces hepatocarcinogenesis in rodents. Fumonisin FB1 is a mycotoxin, capable of contaminating corn worldwide and is associated with various cancers. Fumonisin toxicity may originate from interference with folate metabolism, in HepG2 cells.
Aim: We used a gestational rat model to investigate the impact of methyl-group donors and fumonisin on the pattern of global histone modifications.
Methods: In this study, dams are exposed to methyl donors deficiency and/or fumonisin FB1 and foetuses (E20) are analyzed. We have investigated 4 histone modifications associated with heterochromatin assembly in the liver: H4K20me3, H3K9me3, H3R2me2.
Results: Combined exposition to methyl donor deficiency and fumonisin decreased folate receptor and reduced folate carrier protein, compared to methyl donor deficiency only. Methyl donor deficiency decreased H4K20me3. Combining fumonisin administration to methyl donor deficiency led to an even more significant decrease of H4K20me3 and to an increase of H3K9me3. The elevated H3K9me3 can be viewed as a defence mechanism inciting the cell to resist heterochromatin disorganisation. H3R2me2 varied also according to the defence mechanism hypothesis.
Conclusion and Discussion: Methyl donor deficiency and fumonisin act synergistically to alter selective markers of heterochromatin assembly thus providing a mechanism that can contribute to DNA instability.

P7 04
Can seasonality of one-carbon metabolism in Gambian mothers explain differences in their offspring DNA methylation?

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Background: Animal models show that periconceptual supplementation with folic acid or betaine induces differences in the offspring phenotype, mediated by one-carbon metabolism and DNA methylation. In humans, season of conception in rural Gambia appears to correlate with DNA methylation patterns.
Aims: To investigate i), dietary intake and blood nutritional status of one-carbon-metabolism-related substances in rural Gambia women and ii), possible associations between maternal blood biomarker status and infant epigenetic patterns.
Methods: We followed 30 Gambian women (18-45 y) monthly for one year, to measure dietary intake of choline, betaine, folate, methionine and vitamins B2, B6 and B12 and blood concentrations of those nutrients, as well as SAM, SAH, homocysteine and DMG. We also recruited 180 women conceiving in the rainy or dry season, to assess early pregnancy biomarkers and offspring DNA methylation of previously identified metastable epialleles.
Results: Seasonal patterns were seen for all biomarkers with higher SAM (4.6%), betaine (25.5%), and B2 (12.2%), and lower SAH (-14.2%), DMG (-15.3%), and B12 (-5.6%) levels in the rainy versus the dry season. Dietary intake and epigenetic analyses are underway.
Discussion: The higher SAM:SAH ratio (21.9%) suggests higher methylation potential in the rainy season. Whether this is due to seasonal variation in dietary intake and whether this affects infant DNA methylation patterns remains to be determined; results will be presented at the Leipzig meeting.

P7 05
PABA (B-Vitamin) decreases the level of DSBs in DNA of mammalian blood leukocytes

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Background: Depending on the dosage NO serves the universal signal molecule or extremely toxic agent. Up to date there is no data on the natural compounds being effective regulators of toxic and genotoxic potencies of NO. Our experimental results have shown that PABA (B-Vitamin) was a very strong inhibitor of the SOS DNA repair response and mutagenic processes, induced by alkylation, oxidative and UV- damages in E. coli (Vasilieva S.V.Res. in Microbiol., 2002, V.153).
Aim: The aim of this work was to estimate the efficiency of PABA against DNA damages induced in DNA of mammalian blood leukocytes by NO- donating agents, the potent SOS-inducers.
Methods: The neutral Comet Assay was used to quantify the double-stranded breaks (DSBs) in DNA. The water solutions of crystalline NO-donors - iron-tetranitrosyl complexes (ITNCs, from the Institute of Problems of Chemical Physics RAS) were studied as DSBs inducers in vitro.
Results: It was firstly demonstrated that incubation of the cells with ITNCs leads to a statistically significant dose-dependent linear increase in the levels of DSBs in all experiments. The reliable reducing of DSB's level (up to 70%) was monitored when the cells were incubated with ITNCs in the presence of PABA and wasn't watched if the cell were pretreated with PABA.
Discussion and Conclusion: The DSB-inhibiting phenomenon of PABA can rely on interplay that takes place between NO-donors, intracellular DNA and PABA. We suppose that the decreasing of the total positive charge on DNA being contacted with PABA should decrease the local concentration of positively charged genotoxic ITNCs in the vicinity of DNA target and decrease the DSBs in DNA. In our particular experiments in vitro PABA didn't change the physical and chemical characteristics of ITNCs tested.

P8 – Methodology

P8 01
Novel assays for the determination of biologically active B-vitamins and folic acid

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Question: Higher plasma homocysteine (HC) is associated with a higher cardio-vascular risk. Apart from renal insufficiency, deficiencies of vitamin B6, B12, and folic acid (FA) represent the most common causes of moderately elevated HC.
Aim: We have developed novel, micro-titer plate-based turbidimetric kits (ID-Vit) to determine *biologically active* B6, B12 and FA by measuring bacterial growth in factor-deficient media. This is the first clinical application in patients with coronary artery disease (CAD).
Methods: We collected serum from 18 control patients (C), 13 electively admitted CAD patients (CAD), and 37 patients admitted urgently due to acute coronary syndrome (ACS). We then compared ID-Vit kits with established standard assays for B6 (HPLC determination), B12 (measurement of serum cobalamin, Roche-Elecsys), and FA (Roche-Elecsys method). HC was measured by HPLC.
Results: HC values did not differ between C, CAD, and ACS (15.2 ± 5.9 vs. 14.4 ± 3.4 vs. 14.0 ± 2.7 µmol/l) and were not significantly elevated. Thirty four % of patients had serum creatinine values >1.5 mg/dl. Means of B6, B12, and FA, measured either by standard assay or ID-Vit, did not differ between groups. Individual ID-Vit values were similar to standard values for B6, significantly higher for B12, and lower for FA . ID-Vit data correlated significantly with standard data, with correlation coefficients between 0.58 and 0.66. Neither standard nor ID-Vit values correlated with HC levels.
Conclusions: The first application of ID-Vit assays proved their feasibility and good correlations to standard methods. The lack of correlation between B-vitamins or FA and HC can be accounted for by the fact that HC was in the high-normal range and that a relevant percentage of patients had renal insufficiency.

P8 02
Determination of B-complex vitamins' stability during rye sourdough bread production using LC-MS and stable isotope dilution assay

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Rye sourdough bread is a traditional bread, which is widely consumed in North-West Russia, Baltic countries, Finland, and the North of Germany. Besides other nutritionally valuable compounds it is considered as a good source of B-complex vitamins.
The aim of this work was to study the stability of thiamine, riboflavin, nicotinamide, nicotinic acid, pantothenic acid, pyridoxal and pyridoxine in a rye sourdough bread production.
The concentration of B-complex vitamins were determined in the used raw materials (rye flours, white and red rye malt, yeast) and rye breads using LC-MS and stable isotope dilution assay.
During baking the concentration of vitamins decreased by 40-45% in the case of thiamine, 20-40% in the case of riboflavin, 0-40% in the case of nicotinic acid, 10% in the case of pantothenic acid, 50-60% in the case of pyridoxal and 35-50% in the case of pyridoxine. By contrast, the concentration of nicotinamide increased by 12-19 times. This increase in the nicotinamide concentration was shown to be to result from lactic acid fermentation of the dough by *L. panis* N915 during rye sourdough processing.
In conclusion, the LC-MS combined with stable isotope dilution assay is a promising method for studying B-complex vitamins stability during food processing.

P8 03
UPLC-MS/MS as a Rapid Tool for the Simultaneous Analysis of B-Vitamins in Human Milk

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Introduction: Low concentrations of B-vitamins in human milk due to poor maternal status can result in infant deficiency and associated health risks. Data on the B-vitamin content of human milk is sparse and based on outdated and time consuming methods, but is vital for setting maternal and infant recommended intakes and potentially useful for status assessment.
Aim: Simultaneous, rapid analysis of multiple B-vitamins in human milk.
Methods: We developed a rapid Ultra-Performance-Liquid-Chromatography Mass Spectrometry (UPLC-MS/MS) method to simultaneously analyze thiamin, riboflavin, FAD, nicotinamide and pyridoxal (PL) in human milk. Several sample preparations were tested for suitability and optimized conditions were used for validation.
Results:

Vitamin	DRI	USA	India	p-value
[mg/L]		*mdn*	*mdn*	
Thiamin	0.2	0.03	0.01	< 0.05
Riboflavin + FAD	0.35	0.31	0.14	< 0.05
Nicotinamide	1.8	1.18	0.35	< 0.05
Pyridoxal	0.13	0.03	0.12	< 0.05

Discussion and Conclusion: Best results were obtained after methanol precipitation and removal of non-polar matrix constituents. Analysis of human milk from the US and India revealed that the vitamin concentration differs by geographic origin (p-value < 0.05) and only vitamin B2 (USA) and PL (India) meet the values assumed for setting recommended intakes. Samples from additional countries are being analyzed.

P8 04
Folate forms quantification in serum and whole blood using stable-isotope dilution ultra performance liquid chromatography tandem mass spectrometry

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Background: Low folate status is associated with increased risk of neural tube defects, cardiovascular diseases, cognitive dysfunction,

and cancer. Alterations in folate metabolome might have serious effects on human health.
Aim: We aimed at developing an ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the quantification of 5-methyltetrahydrofolate (5-methylTHF), 5-formylTHF, 5,10-methenylTHF, THF, and folic acid in serum and whole blood (WB) hemolysates.
Methods: We describe an UPLC-MS/MS method for the quantification of key folate forms. After an Oasis MAX solid-phase extraction step, folates were eluted by methanol containing 1% formic acid. Dried eluates were resuspended in H_2O/methanol (60:40, v/v) containing 0.1% formic acid and 1 g/L ascorbic acid. Sample separation and measurement was performed within 2.5 min.
Results: We observed quantitative oxidation of THF to folic acid in WB. Due to interconversions, WB folate was summarized as 5-methylTHF and non-methylTHF. The method was linear over 0.2-200 nmol/L ($r^2 \geq 0.9999$). In serum and WB, limits of detection were between 0.119-0.669 nmol/L. Interassay CVs were between 2.8-15.4% for the different folates. In non-supplemented adults median (10^{th}-90^{th} percentile) 5-methylTHF concentrations in serum samples (n=32) were 15.9 (5.6-26.7) nmol/L and THF 2.07 (<LOD-4.05) nmol/L. 5-MethylTHF in WB samples (n=48) was 445 (240-955) nmol/L in addition to 71.2 (47.9-129) nmol/L non-methylTHF.
Discussion and Conclusion: Our UPLC-MS/MS method has high sensitivity and selectivity for the folate quantification. The fast sample preparation and analysis allows the use in large-scale clinical studies. THF oxidation to folic acid can give wrong estimation of folic acid in WB.

P8 05
Plasma phospholipids quantification by ultra performance liquid chromatography tandem mass spectrometry

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Background: Phospholipids (PL's) are important components of cell membranes and Lipoproteins. Changes in the concentrations PL's have been observed in cancer, dementia and diabetes. PL's might be biomarkers related to the methylation cycle via the PEMT pathway.
Aim: To develop a new method for the quantification of PL's in human plasma.
Methods: The presented UPLC-MS/MS method utilises two external calibrators and one internal standard for each class. The chromatography takes 15 min. To compare the method with others we analyzed the plasma of 34 apparently healthy volunteers (eight males, age 24-80 years).
Results: Mean concentrations (SD) in apparently healthy people were: total phosphatidylcholine (PC) 2020 (376) µM, total sphingomyelin (SM) 399 (77) µM, total lysophosphatidylcholine (LPC) 109 (36) µM and total phosphatidylethanolamine (PE) 27 (15) µM. We quantified 41 individual PL species. The relative concentration of the species in healthy volunteers was comparable with the literature. Inter-assay coefficients of variation were <10% for the most abundant species and <20% for all quantified PC, LPC, and SM species and the three most abundant PE species. Quantification was linear over the physiological ranges for PE-, LPC-, and SM- and up to 500 µmol/L for PC-Species. Coefficients of linear regression were R^2>0.98. Mean recoveries were between 83% and 123%. The limits of detection were 0.37 µmol/L for PC, 0.86 µmol/L for SM, 4.02 µmol/L for LPC and 3.75 µmol/L for PE.
Conclusion: We present a UPLC-MS/MS method for the quantification of total PC, PE, LPC, and SM and several species within these classes in EDTA-plasma. The method is simple, fast, simple and robust and it complements the available range of validated methods.

P9 – Molecular and cellular mechanisms

P9 01– WITHDRAWN

P9 02
Evaluation of the genotoxicty of three isoflovone phytoestrogens in wing spot test of D. melanogaster using mutant strains and role of folic acid in genomic stability of somatic cells

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In this study, the potential genotoxic effects of three different phytoestrogens (genistein, quercetin and glisitein) whose biological effects bear similarities in natural and cultured plants which are used in feding animals and human beings, and antimutagenic effects of folic acid were researched via using *Drosophila* wing somatic mutation and recombination test (SMART)(1). In the exrepiments, larvae of 72±4 hours trans-heterozygote which have the *mwh/flr*^{3} genotype of *Drosophila melanogaster*, were used. We worked on four concentrations of each of the 3 phytoestrogens whose genotoxic effects were researched. The larvae were fed chronically with *Drosophila* instant medium containing different concentrations of phytoestrogens. The effects of phytoestrogens used were evaluated considering the mutant trichomes.
In the second stage of our study, we applied by far the highest dosage of these phytoestrogens, which is 10mM, together with the 10mM dosage of folic acid on the larva in the same feeding environment. The wing preparates of the obtained individuals were prepared for *mwh/flr*^{3}(normal wing phenotype) and *mwh/TM3* (serrat wing phenotype) genotype.
As a result of the applications glisitein yielded in all concentrations either negative or inconclusive genotoxicity. However the other two phytoestrogens on the whole showed positive genotoxicity depending on the increase in concentration. Total mutation frequency for normal wing phenotype the order of the genotoxicity was: quercetin 0.89%, genistein 0.58% and gilisitein 0.19%. When these phytoestrogens were applied together with the folic acid, total mutation frequency decreased, respectively for kuersetin 0.46%, genistein 0.28% and gilisitein 0.16%. The negative or positive genotoxic effects of the plant estrogens used in this study were determined and it was found out that folic acid is anti-genotoxic against these effects.

P9 03
Methyl donor status influences global DNA methylation and DNMT expression in ervical cancer cells

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Background: Epidemiological studies suggest that folate may modulate cervical cancer risk and progression. This effect may

be mediated by altered DNA instability and aberrant DNA methylation.
Aim: To develop a model of folate and of folate and methionine depletion of cervical cancer cells in culture; to examine effects on global DNA hypomethylation and expression of DNA methyl transferases (DNMTs), and to determine whether any such effects are reversible.
Methods: The human cervical cancer cell line, C4-II, was grown in folate and methionine-replete, folate-depleted, and folate and methionine-depleted medium. Intracellular folate and homocysteine export were measured. DNA global methylation status of cells was investigated using a flow cytometric method.DNMT expression was determined using RT-PCR. A minimum of three independent experimental replicates were carried out.
Results: We have generated a cervical cancer cell model of methyl donor depletion characterised by low intracellular folate, accumulation of homocysteine and enhanced export. Combined depletion of folate and methionine led to an 18% reduction in global DNA methylation. Folate and methionine depletion resulted in a significant down-regulation of DNMT3a and DNMT3b, showing a 2.63 and 3.57-fold reduction in expression respectively, after 8 days of depletion. Effects on DNMT1 expression were less consistent, but nevertheless a mean 1.75-fold reduction in expression was seen by 8 days of depletion. Early results suggest that these effects are reversible.
Discussion and conclusions: Methyl donor status may influence cervical cancer risk and progression, through effects on DNA methylation, perhaps via modulation of DNMT expression.

P9 04
Effect of methylation on amyloid precursor protein (APP) in Down syndrome fibroblasts

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Background: Dementia is a major health problem world wide. Neuritic amyloid plaques are typical neuropathological hallmarks of AD. Amyloid in senile plaques is the product of cleavage of the amyloid precursor protein (APP) by a series of proteases, β- and γ-secretases. Epidemiologic studies confirmed an association between elevated concentrations of total homocysteine (tHcy) and cognitive dysfunction. Folate, vitamin B12 and B6 are important cofactors for key enzymes that mediate Hcy metabolism. Patients with Down syndrome have additional copies of APP and cystathionine beta synthase genes, thus combining disturbances in the methylation cycle and increased dementia risk.
Aim: We aimed at testing the link between disorders in one carbon metabolism and protein levels of full length APP and its by-product C99.
Methods: Down syndrom fibroblasts were incubated either in a vitamin-rich medium or in medium free of vitamins B12, B6 and folate. Different concentrations of Hcy, S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM), or lovastatin were added for 24 hours. Protein expressions of APP and C99 in cell extracts were investigated by western blot. Results were normalized for β-actin.
Results: Hcy did not affect and SAH increased total APP in cell extracts. SAM caused reduction of APP protein expression only in cells grown in a vitamin-free medium. Lovastatin lowered APP in cells incubated in a vitamin-rich medium. Inhibitors of b- and γ-secreatases reverted the effect of SAH, SAM and that of lovastatin on protein expression of APP and the effect of Hcy on C99. Inhibitors of b- and γ-secreatases reverted the effect of SAH and that of SAM on protein expression of APP and the effect of dLHcy on C99 protein.
Conclusion: Alterations in methylation conditions in Down syndrome fibroblasts caused changes in key proteins in the amyloidogenic pathway. Furthermore, the possible effect of lovastatin against dementia seems to depend on B-vitamins status.

P10 – Other topics

P10 01
Effect of Nicotinamide on Experimental Induced Diabetes

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Insulin dependent diabetes mellitus (IDDM) results from irreversible loss of beta cells (β-cells) of the pancreas. A Streptozotocin (STZ)-induced diabetes in animal model mimics, in some aspects, recent onset IDDM. This study was conducted to investigate the effect of nicotinamide on experimentally-induced IDDM. Thirty Spraque Dawley rats were divided into 3 groups; a control group, a diabetic group which received an intraperitoneal (i.p.) injection of 55 mg/kg STZ and a nicotinamide group (1g/kg/day) which were dosed orally for 3 days followed by (i.p.) STZ (55 mg/kg) with the nicotinamide treatment continuing for an additional 14 days. Rats receiving STZ became diabetic after 2 weeks. This diabetic group showed hyperglycemia, and a very low level of C-peptide.
Furthermore, pancreatic islets exhibited increased nitric oxide (NO) production together with an increased apoptotic index (as detected by TUNEL and electron microscopy). Nicotinamide treatment prevented STZinduced diabetes, it also antagonized an increase in NO, and inhibited β-cell apoptosis. Fasting blood glucose, serum insulin and serum C-peptide were all within the normal range in the nicotinamide group. The nicotinamide protection of β-cells may be facilitated via inhibition of apoptosis and nitric oxide generation. It is suggested that nicotinamide might be considered an effective agent for the prevention and treatment of IDDM in prediabetic, and early stages, of IDDM.

P10 02
Potential role of dietary folate and choline as radio-protective agents

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Background: Radiation exposure poses a major risk for workers in the nuclear power plants and other radiation related industry. Folate and choline might be of radio-protective value as they are, within broad dose ranges, non-toxic to humans.
Aim: *1)* To investigate choline dependent adaptive response to cytotoxic effect of folate deficiency and gamma (γ)-radiation. 2) To demonstrate that γ-radiation is an efficient DNA demethylating agent and its injurious effect can be minimized by dietary methyl supplements (folate, choline and vitamin B12). 3) To examine the effect of γ-radiation on choline and choline containing moieties in choline deficient subjects.

Methods: Male Swiss mice maintained on various combinations of folate and choline sufficient and deficient diets, were subjected to total body γ-irradiation. It was followed by investigation of the profile of downstream metabolites and activity of one-carbon (C_1) flux generating enzymes. Liver and brain samples were also subjected to histo-pathological examinations.
Results: 1) Folate deficiency modulated purine/pyrimidine free sites in DNA 2) Modification in levels of folate and choline metabolism regulatory enzymes, 3) Release of choline reserves from liver to serum and 4) Enhanced C1 flux towards DNA methylation.
Conclusions: 1) Folate deprivation and γ-radiation interacted to mobilize additional choline reserves of hepatic tissue, for redistribution to other organs 2) Maintenance of genomic DNA methylation under γ-radiation stress involved increased one-carbon flux through various metabolites. 3) Interaction between choline deficiency and γ-radiation substantially enhanced liver adipogenesis.

P10 03
Does SAMe Hurt Your Heart - A Literature Review

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Background: S-adenosyl-L-methionine (AdoMet, SAMe), a dietary supplement has been studied for treatment of depression, liver cirrhosis, degenerative joint disease, fibromyalgia, and neurologic disorders. AdoMet is metabolized to S-adenosyl-L-homocysteine, which can then be hydrolyzed to homocysteine (Hcy) and adenine. This pathway raises the possibility that individuals who take AdoMet could develop elevated levels of Hcy, a potential unfavorable outcome.
Aim: To determine if exogenous AdoMet increases plasma levels of Hcy.
Methods: Literature review.
Results: We located 3 studies:

i. Loehrer et al (1997) studied the effect of one oral dose of 400 mg AdoMet on plasma Hcy in 14 healthy human subjects over a 24 hour period. Plasma Hcy did not change significantly.
ii. Gören et al (2004) reported on 15 healthy human subjects who received oral AdoMet 1,600 mg/day for 4 weeks. No patient developed increased Hcy levels.
iii. Thompson et al (2009) performed a placebo-controlled, double-blind, randomized trial involving 52 healthy human volunteers receiving placebo or AdoMet (800 mg/day for 4 weeks). AdoMet did not significantly affect plasma Hcy levels.

Discussion and Conclusion: The studies described were performed on healthy human subjects over a short period of time. Changes in Hcy may take place over a longer period of time and may be different in patients with elevated Hcy levels. Future clinical trials are therefore needed. The results of an ongoing trial at Baylor (NCT00473200) evaluating the effect of oral AdoMet (1200 mg/day) for 6 weeks with and without supplementation of folate, vitamin B12 and B6, on plasma Hcy levels in patients with vascular disease and moderate hyperhomocysteinemia should help to assess the effect of AdoMet use.

P10 04
Purification and Characterization of Acidic Protease from *Aspergillus oryzae* BCRC 30118

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Background: Acid proteases are frequently used in the production of seasoning materials, protein hydrolysate, soy sauce, or used as digestive aids. They are also widely used to improve the texture of flour paste, to tender the fibril muscle, and to clear beer and fruit juice. Many free amino acids and peptides are produced during fermentation of soy source, as a result of the hydrolysis of soybean protein by various proteolytic enzymes produced by *Aspergillus oryzae*. However, the properties of these enzymes have not yet been completely elucidated.
Aim: This study aimed to purify and characterize the acid protease produced from *Aspergillus oryzae* BCRC 30118.
Methods: Amicon ultrafiltration (cutoff: 10 kDa), DEAE Sephacel and Sephacryl S-200 HR chromatographs were used to purify the acidic protease from 3-day cultivation of *Aspergillus oryzae* BCRC 30118 at 25°C. The purity and molecular weight were determined using SDS-PAGE. Substrate, metal ions and inhibitor effects were assayed. The enzyme kinetics were also determined.
Results: The specific activity, yield and purification fold were 121.61 kU/mg, 15.1% and 6.9 fold, respectively. The MW of purified acidic protease was 41.0 kDa estimated by SDS-PAGE, while the optimal pH and temperature were 3.0 and 60°C, respectively. It was stable at pH 3.0-6.0 and 4-35°C, respectively, inhibited by Fe^{2+}, Hg^{2+}, Fe^{3+} and pepstatin A, and slightly by leupeptin and TPCK.
Discussion and Conclusion: According to the substrate specificity and inhibitor study, it was considered to be chymotrypsin-like protease. The activation energy was 37.5 kcal/mol, while the K_m, V_{max} and K_{cat} for the hydrolysis of hemoglobin were 0.12 mM, 14.29 μmol/min and 14.55 sec^{-1}, respectively.

P10 05
Nucleocytoplasmic distribution of hepatic methionine cycle enzymes in hepatopathy

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Background: Enzymes of the methionine cycle have been traditionally identified in the cellular cytoplasm, but recent reports described some of these proteins also in the cell nucleus. This is the case of S-adenosylhomocysteine hydrolase (SAHH), glycine N-methyltransferase and, more recently, of methionine adenosyltransferases (MATs) I/III and II. Mechanisms that regulate the nucleocytoplasmic localization of these enzymes and its putative relationship to disease remain unknown.

Aim: The present work was designed to gain insight into the mechanisms that regulate localization of methionine cycle enzymes, and to investigate whether D-galactosamine induced hepatopathy associates with changes in nuclear levels.
Methods: Confocal microscopy, immunohisto-chemistry, subcellular fractionation, western blot, activity measurements, and gel filtration chromatography are the main methods used to determine the changes derived from the treatment.
Results: Our results indicate a role for oxidative stress in nuclear localization of MAT I/III and SAHH and their nuclear accumulation upon D-galactosamine treatment. No changes in SAHH oligomeric state were detected in the nucleus, whereas an increase in MAT activity together with a boost in the amount of MAT I was observed upon comparison of nuclear profiles from control and galactosamine-treated rats.
Discussion and Conclusions: Altogether these results suggest a higher need of nuclear AdoMet production and AdoHcy elimination during hepatopathy. Such a need might be related to epigenetic changes affecting trimethylation of H3K27 preferentially, the main epigenetic modification previously described as altered by nuclear accumulation of MAT I/III[1].
[1]Reytor et al. (2009) FASEB J 23, 3347-3360.

P10 06
Short-term creatine supplementation does not reduce increased homocysteine concentration induced by acute exercise in humans.

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Background: Studies in rats have shown that creatine supplementation modulates the flux of transmethylation reactions and decreases blood homocysteine (Hcy) levels. However, this effect in humans is poorly known, specially after acute exercise.
Aim: To evaluate the effect of creatine supplementation on Hcy levels after acute exercise in humans.
Methods: Twenty-three sub-20 soccer players were divided 2 groups: creatine supplemented (Cr) and placebo (Pla). The supplementation was performed in double-blind controlled manner using creatine or placebo tablets with 3 g/kg during 7 days. Before and after 7 days of supplementation, the athletes performed an acute high-intensity anaerobic exercise (two consecutive RAST protocol consisted in 6 x 35 m sprint with 10 s between them). Blood samples were collected before and after 7 days supplementation as well as 0 h and 1 h after exercise protocol.
Results: Plasma creatine concentration was significant increased ($P < 0.05$) (Pla 107.5±10.2 vs Cr 330.2±52.3, umol/L). Controversially, creatine supplementation did not change Hcy after 7 days supplementation (Pla 6.9±0.2 vs Cr 7.2±0.2, umol/L) nor after acute exercise (Pla 8.2±0.3 vs Cr 8.4±0.3, umol/L). Hcy concentration significant increased ($P < 0.05$) 1 h after acute exercise (18%). Acute exercise also decreased red blood cell SAM 30%, but without changes in SAM/SAH ratio. Also, it was not found changes in plasma concentration of vitamin B12 and folate as well as cysteine and methionine.
Conclusions: Acute anaerobic intense exercise increased Hcy concentration in plasma. However, creatine supplementation did not decreased plasma Hcy induced by exercise. These results are different from experiments in rats.
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P10 07
Inhibitory effect of vitamin B6, pyridoxal 5′-phosphate on lymphangiogenesis

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Background: Accumulating evidence suggests that consumption of vitamin B6 may lower the risk of colorectal cancer. As the mechanisms, reducing cell proliferation, angiogenesis and inflammation have been demonstrated. However the mechanisms have not been fully understood yet. Lymphangiogenesis, formation of new lymphatic vessels, is involved in inflammation, which is linked to tumorigenesis. We hypothesized that the suppressive effect of vitamin B6 on colorectal tumorigenesis might be due to in part anti-lymphangiogenic effect.
Aim: The aim of this study is to clarify the effect of vitamin B6 (pyridoxal 5'-phosphate; PLP) on lymphangiogenesis.
Methods: The effect of PLP was evaluated in ex vivo lymphangiogenesis model, in which rat thoracic ducts were isolated and cut ducts (lymphatic rings) were cultured in collagen gel. In addition, we examined the effect in vitro models using human lymphatic endothelial cells (LYECs).
Results: PLP suppressed outgrowth of microvessels from lymphatic ring in ex vivo lymphangiogenesis model, showing PLP has anti-lymphangiogenic activity. PLP also inhibited LYEC tube formation and proliferation.
Discussion and Conclusion: In this study we clearly show that PLP suppress lymphangiogenesis in both ex vivo and in vitro lymphangiogenesis models. PLP exerts its inhibitory effect through suppressing LYEC tube formation and proliferation. The inhibitory effect of vitamin B6 (PLP) on lymphangiogenesis might in part lower the risk of colorectal cancer.

P10 08
Riboflavin deficiency inhibits the viability and activity of macrophages RAW 264.7

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Background: Riboflavin as a vitamin is necessary for the proper functioning of our body. Its level in plasma is mainly conditioned by the content of the diet. Therefore, we can observed states of its deficiency (3.1 nM in plasma) or enrichment in case of tablets taking (300 nM).
Aim: The aim of this study was to investigate the effect of riboflavin concentrations on the viability and activity of macrophages.
Methods: Studies was performed on RAW 264.7 cells, cultured in customized medium with riboflavin concentration 3.1; 10.4 or 300 nM, supplemented with antibiotics (1%) and fetal bovine

serum (10%). Cells were cultured for up to 4 days in presence or absence of LPS (1 ug/ml, for the last 24 hr). We investigated cell viability, and activity (cell cycle progression, cell adhesion, respitatory burst, phagocytosis), and cell activation (nitric oxide and cytokine/chemokine released, and iNOS, TLR4, CD14 production).

Results: We found that culturing of RAW 264.7 cells in low riboflavin content (R; 3.1 nM) leads to inhibition of proliferation, increased cell death by apoptosis, and to disruption of cell activity. Moreover riboflavin deficiency caused impairs of cells response to LPS activation by reduction of TLR4 and iNOS expression, affected cytokine/chemokine production as well as nitric oxide released.

Discussion and Conclusion: The present study provided evidence that RAW 264.7 cells are sensitive to changes of riboflavin concentration and show signs of its deficiency within only 4 days. Inhibition of macrophages activation by riboflavin deficiency can lead to the breakdown of innate immune defense and increased the susceptibility to infections.

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P10 09–WITHDRAWN

P10 10–WITHDRAWN

P10 11

Alcohol increases homocysteine concentration in Nepalese Population: A Hospital Based Observational Study from Western Nepal

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Background: Alcoholic liver disease (ALD) is a common disorder which causes serum liver enzyme elevation. Elevated homocysteine levels was demonstrated in Alcoholic liver disease and chronic liver failure.

Objective: To find out whether there is an association between homocysteine levels and ALD.

Methods: 128 patients with ALD and 317 healthy adults enrolled in the study. Fasting blood samples were obtained and serum homocysteine levels were measured by fluorescence polarization immunoassay (FPIA) technology. Oral glucose tolerance test was performed and serum insulin, c-peptide, and lipoprotein levels were also measured.

Results: The mean serum homocysteine levels (+/-SD) were 14.94±2.10 µmol/L and 13.12±1.01 µmol/L in ALD and the control group, respectively. Mean serum homocysteine level in the ALD group was significantly higher than in control group ($p<0.01$). Fasting blood glucose, insulin, total cholesterol and low density lipoprotein (LDL) cholesterol were all found higher than the control group.

Conclusion: Non invasive tests have demonstrated a reasonable ability to identify significant fibrosis, cirrhosis in particular, nor is it surprising that liver disease specialists and patients favour a non invasive approach. The serum homocysteine levels were significantly higher in patients with ALD than in control group. This may point out that high homocysteine levels may be associated with ALD.

P10 12

Lipid Rafts Cross-talk with Toll-Like Receptors (TLRs): relevance to the CONVERSION of PrPc to PrPSc in Prion Disease Pathogenesis

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Direct evidence has been collected regarding the involvement of inflammation and unregulated aggregation of proteins in the pathogenesis of a range of neurodegenerative disorders such as Alzheimer disease, Morbus Parkinson and prion disease. Inflammatory signaling is generally triggered by the cytokine-responsive tumor necrosis factor (TNFR) and toll-like receptor (TLR) super families. Prion protein is a cell-surface glycosylphosphatidylinositol (GPI)-anchored protein that is normally expressed in neurons, various non -neuronal tissues and leukocytes. Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal neurodegenerative diseases in mammalian species that are sporadic, but also have been traced to mutations and to infectious transmission, including iatrogenic transfer. TSEs include kuru, Creutzfeldt-Jakob disease (CJD), Gerstemann-Sträussler syndrome (GSS), and fatal familial insomnia (FFI) in human beings, as well as scrapie in sheeps and goats, bovine spongiform encephalopathy (BSE) in cattle, and encephalopathies in mink, cats, mule, deer, elk, and several exotic ungulates. Neuron loss, spongiform degeneration and glial proliferation are the main pathological consequences of TSEs. The exact nature of the causative agent for TSE has yet to be fully determined. However, it is widely believed that an abnormal form, (PrPSc) of a host cellular prion protein (PrPc) may compose the substantial parts of the infectious agent. At present there is no treatment or cure for prion disease. Extensive data from literature indicates that the fundamental event in TSE disease is the conversion of the normal, detergent-soluble, proteinase K-sensitive isoform or prion protein, PrPc to an abnormal, detergent-insoluble, partially proteinase K-resistant isoform, PrPSc, and the accumulation of this abnormal isoform in the central nervous system of infected animals. Despite enormous investigative work on prion diseases, the mechanisms underlying the process of PrPc to PrPSc conversion remain incompletely understood. However, heparan sulfate, heat shock proteins, and glycosylphosphatidylinositol (GPI)-anchor and specific RNA molecules have been argued to be implicated in this process. In addition, findings from work in cell biology and biophysical studies suggest an important role for lipid rafts in PrPc to PrPSc conversion. Although signal transduction activity of lipid rafts is well established, limited information is available regarding the signaling pathways and the nature of receptors responsible for both cell to cell interactions, and the biological effects of the cells embedded in the lipid rafts in the process of PrPc to PrPSc conversion. PrPc, for instance, being a GPI-anchored protein, lacks a cytoplasmic domain and thus cannot interact directly with cells on the cytoplasmic face of plasma membrane. In particular, how the activation signals mediated by PrPc (a GPI-anchored glycoprotein), are transmitted across the plasma membrane remains unclear. Now, recent advances in immunology have disclosed the presence of a family of transmembrane protein, termed, Toll-like receptors (TLRs). The TLRs are important sensors of the innate immune system that serve to identify conserved microbial components, ranging from lipids, lipoproteins, proteins and nucleic acids and also non- microbial motifs such as heparan sulfate, heat shock proteins, small synthetic molecules and cell-injured molecules. In line with the aforementioned, and coupled with the findings that similar to PrPc, many engulfing receptors including, CD36 (a sensor of diacylglycerides: TLR-2 ligands), CD44, CD14 (a prominent

co-receptor with TLR4) are present in lipid rafts, and the fact that despite the diversity of GPI-anchored molecules found in parasitic protozoa, fungi and mammalian cells, all eukaryotic GPI-membrane anchors have a common core structure consisting of a conserved trimannosylglucosaminyl glucan(Manα1,2-Manα1,6-Manα1,4-GlcN-,) linked to the inositol residue of a phosphatidylinositol(Man3-GIN-PI) and the recent findings that TLRs mediate the activity of a G protein-coupled receptor formyl peptide receptor like 1 (FPRL1),which had been reported to mediate binding activity of $PrP_{106-126}$ (PrPSc peptide mimetic), I am tempted to suggest that Toll-like Receptors might have an important role in driving the process of PrPc to PrPSc conversion in prion disease pathogenesis. Proactively addressing this subject may highlight new avenues for future investigation into the prevention, and possibly, the treatment of TSEs and related diseases.

P10 13

MORBUS ALZHEIMER and Human Cartilage Glycoprotein-39 (HC-gp39) : relevance to the Pathology of Alzheimer's Disease and Disease Management–Dedicated to the Memory of Prof. Mark Anthony SMITH: a Unique Colleague

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Abundant evidence now exists indicating that oxidative damage to macromolecules, dysregulated inflammatory responses, endothelial dysfunction and aberrant immune/chaperone alterations play important roles in the neurodegenerative processes in many neurological diseases including Parkinson's Disease, Creutzfeldt-Jakob Disease and Morbus Alzheimer. Another important phenomenon in these lesions is deregulated autophagic process. Alzheimer's disease (AD), Multiple Sclerosis, Huntingston' disease, Morbus Parkinson and Creutzfeld-Jakob Disease (CJD), are all neurodegenerative disorders (ND) in humans, which terminate in untimely death in the final resolution of disease development and progression. The eatiology of these disorders is unknown and there is presently no drugs to completely arrest the progression of the associated neurodegenerative processes. Alzheimer's disease, apparently the most widespread ND associated with aging, results in a progressive loss of cognitive function and dementia, the prevelance of which increases exponentially with aging and thus, represents a serious public health burden worldwide. Precise mechanism(s) that underpin neurodegeneration in AD brain is not completely understood. However, converging evidence derived from postmortem brain from Alzheimer's disease patients and transgenic mouse model of AD suggest that oxidative stress, disordered inflammatory response and deregulated autophagy are associated with mitochondria in early AD progression. Amyloid beta (Aß) and amyloid precursor protein, and tau are known to localize to mitochondrial membranes block important transportation routes to the mitochondria,disrupt the electron transport chain,enhance the generation of reactive oxygen species, bring about mitochondrial damage and thus, prevent normal neuronal function. Additionally, in commensurate to this, accumulation of amyloid beta (the most proteinacious molecule that characterizes plagues in the brain of individuals with AD) and tau at synaptic terminals might contribute to synaptic damage and, thereby, to 2cognitive decline in patients with AD. These further buttress the concept that these dysfunctions (oxidative stress, deregulated inflammatory responses and defective autophagy), working in concert and/or independently, are potentially detrimental to AD patients. A plethora of inflammatory mediators and macrophage-derived molecules have been associated with AD pathology. Now, emerging large body of literature associated exaggerated serum levels of HC-gp39 with severity of disease processes in a range of neurodegenerative diseases and many other human disorders characterized by inflammation and endothelial dysfunction, thereby indicating that HC-gp39 is present in the brain and also participates in the pathogenesis of the disorders. The hypothesis that HC-gp39 is implicated in the pathogenesis of neurodegenerative disease was first proposed in 1997 (Okom Ofodile, PhD-thesis) and has subsequently been proven by a variety of experiments (Rudy Castellani et al 2005, Tsuji et al 2002,) including genetic methods (Riemer et al. 2000). HC-gp39, a heparin and collagen-binding glycoprotein and a chitin-binding lectin, is a pathogenesis-related protein and, a mammalian chitinase-like protein without enzymatic activity. The physiological and pathophysiological role of HC-gp39 and the cellular receptors critically underlying its physiological effects remain enigmatic. Perhaps most importantly, the mechanisms by which HC-gp39 mediates its biological effects is almost completely unknown. In this context, unraveling the mechanism(s) by which HC-gp39 exerts its biochemical effects will be instrumental in understanding the pathophysiology of a plethora of human disorders including Alzheimer's disease and CJD. Now, after scrutiny of the work of Annaliese. Recklies et al (2002, 2004) and, studying the havoc associated with high serum levels of HC-gp39, a Hypothesis was formulated in 2010 (Okom Ofodile) : HC-gp39 may mediate its biochem. effects by: 1) Modulation of autophagic program, 2) Direct and indirect activation of the complement, 3) Triggering ROS generation (contribution of oxidative stress could lead to vicious circles as it impinges upon mitochondrial dysfunction, excitotoxicity, lipidoxidation, and inflammation), 4) perturbation of TRIF regulatory function (TRIF is an Adapter protein that transduces signal from Toll-like Receptor 4(TLR4) and TLR3, 3permits the induction of many cytokines, including interferon beta which signals Type I interferon receptor) function- leading to significant amplification of the inflammatory environment. 5) Triggering accelerated hyperimmunity of autoimmunity, and 6) Induction of "Autotoxicity: a phenomenal process first described by McGeer PL/McGeer EG. Autophagy (Greek word for "Eating oneself") is an evolutionarily conserved homeostatic process, whereby cytosolic components are targeted for removal or turnover in membrane-bound components (autophagosomes) that fuse with lysosome. Autophagy process regulates the turnover of damaged organelles and long-lived proteins that are too large to be delivered to the proteosome. Defective autophagy has been mechanistically linked to cancer, neurodegenerative diseases, myodegeneration and cardiomyopathy. Given the emerging functions of autophagy in immune cell homeostasis, immune cell activation and impacts in oxidative and inflammatory cascades, coupled with the hovac associated with high levels of HC-gp39,it seems reasonable to contemplate that the ability of HC-gp39 to impact upon autophagic machinery (Okom Ofodile), might represent the central mechanisms through which HC-gp39 affects its biological Effects (Okom Ofodile, 2010). The above considerations indicate that HC-gp39-mediated activities are critical in AD pathogenesis and should be significant in evaluating anti-inflammatory and antioxidant therapy for Alzheimer's disease. Further, HC-gp39 has the ability to display biphasic dose response (Hormesis) and, because the hormetic biphasic response relationship of HC-gp39 may constitute excellent putative therapeutic targets, functional characterization of the "Dose-Response Relationship" ("the overlapping phase") may have great potential for drug discovery. Thus, delineating the role of specific signaling pathways governing the expression of HC-gp39 and the specific signaling pathways governing the expression of HC-gp39 and the specific signaling pathways that underpin the Crosstalk between HC-gp39 and autophagy during inflammatory processes may yield therapeutically relevant insights to AD disease and many other degenerative disorders characterized

by inflammation, endothelial dysfunction and deregulated autophagy. Here, we discuss the possible 4role of HC-gp39 in the development and the resolution of the pathology of Morbus Alzheimer and alongside suggest possible therapeutic implications.

P10 14
A cohort study of 359 Chinese patients with methylmalonic aciduria in Mainland China

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Background: As the improvement of the medical technology, the number of the inherited metabolism disorders that could be screened and treated is increasing. Methylmalonic aciduria is the most common disorder of organic acidurias in China. Early diagnosis and adequate treatment contributes a lot to improve the prognosis of the patients.
Aims: To investigate the profile of 359 Chinese patients with methylmalonic aciduria in Mainland and to emphasize the importance of the neonatal screening to the treatable congenital metabolism disorders.
Methods: From 1996 to 2011, a total of 359 patients from 19 provinces or cities of Mainland China were diagnosed in our hospital by urine organic acids analysis using GCMS. Serum and urine total homocysteine were determined using a fluorescence polarization immunoassay.
Results: 248 of the 359 patients had combined methylmalonic aciduria and homocysteinemia (69.0%). Isolated methylmalonic aciduria was found in 111 cases (31.0%). 324 cases came from the 11 northern provinces or cities of Mainland China. Among them, 92, 71 and 47 were referred from Hebei, Shandong and Beijing. 35 patients came from southern China.
Discussion and Conclusion: Combined methyl-malonic aciduria and homocysteinemia is a common type of methylmalonic aciduria in Mainland China. Serum and urine total homocysteine determination is essential to distinguish isolated methylmalonic aciduria and combined type. Neonatal screening programs should be expanded in China for methylmalonic aciduria and other treatable metabolism disorders to reduce the morbidity and mortality. Nation-wide study is important.

P10 15 – WITHDRAWN

P10 16
Haptocorrin, transcobalamin and CD320 in liver cancer patients, before and after abaltive treatment

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Background: The cobalamin binding protein, haptocorrin (HC) has shown promise as a tumor marker in patients with fibrolamelar hepatocellular carcinoma, but little is known concerning its concentration in other types of HCC.
Aim: To explore whether HC and the other cobalamin binding protein, transcobalamin (TC), including its holoform (holoTC) and its receptor (CD320) would be suitable as a tumor marker in patients with HCC.
Methods: Blood Samples were collected from 42 patients diagnosed with HCC, before and 1, 4 and 12 weeks after ablative treatment of the tumor. The total level of HC, holo- and total TC and the TC receptor CD320 was analyzed by in-house assays.
Results: We report 13 patients to show values above the interval of reference (240-630 pM) for HC, but none displayed values above 1100 nmol/L. TC was within the interval of reference (500-1500 pM) for all but four patients. One patient with cryptogenic cirrhosis showed markedly increased TC values of 17000 pM. Surprisingly, holoTC displayed markedly increased levels in 13 patients. CD320 displayed relatively low values except for two patients showing markedly increased values. None of the parameters showed systematic changes following ablation treatment.
Discussion/conclusion: None of the biomarkers proved suitable as tumor markers for HCC. However, changes in one or the other of the cobalamin related proteins were observed in more than half of the patients, an observation that warrants further studies in order to understand the changes in cobalamin metabolism in relation to liver diseases.

P10 17
The Effect of Choline Administration in Normal and Diabetic Rats Using 99mTc Tin Colloid (TIN) and 99mTc Mebrofenin (BrIDA) Functional Liver Imaging

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To investigate the effect of choline administration in experimentally-induced diabetes mellitus (DM) in the rat using 99mTc Tin Colloid (TIN) and 99mTc mebrofenin (BrIDA) liver functional imaging.
Methods: Four groups of rats (total n=80, n=20 each group) were studied for both TIN and BrIDA imaging: group C (control), group D (diabetic untreated), group C/Ch (control treated with choline) and group D/Ch (diabetic treated with choline) for 2 weeks.Imaging was obtained in each group and 2 weeks after induction of DM using streptozotocin (55 mg/kg ip) or choline administration (5 g/kg ip). Dynamic acquisition was performed for 1 h after injection of 37 MBq TIN or BrIDA. Organ distribution was determined by drawing regions of interest then obtaining ratios as cumulative count rate over heart or liver or spleen to WB for TIN and liver, liver parenchyma, biliary tree, or abdomen to WB for BrIDA for all groups. Statistical analysis was done using Student's t-test and ANOVA.
Results: TIN uptake ratios (mean±SE) showed a highly significant lower liver uptake (0.73±0.01) in D as compared to C (0.81±0.01). Choline administration showed highly significantly difference between D and D/Ch and C/Ch that brought back the diabetes liver uptake ratio in D/Ch (0.80±0.001) to control levels. While the cardiac blood pool and spleen showed significant higher uptake in the D group rats vs controls. Choline significantly returns the cardiac blood pool (0.17±0.005) to control levels (0.15±0.006) as well as spleen uptake ratios in D and D/Ch and C/Ch. Choline administration has no effect on BrIDA uptake ratios on diabetes.
Conclusions: Choline has detectable changes in the liver phagocytic function after induction of DM compared to controls.

P10 18
Lethal thiamine deficiency in several bird species in the Baltic Sea

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Thiamine deficiency has recently been demonstrated in several species of wild birds, both adults and newly hatched young, in connection with extensive mortality in a paralytic disease in the Baltic Sea. This thiamine deficiency may be the dominating cause of the observed population declines in many bird species during the last three decades. Accordingly, the need for an understanding of this problem is urgent. Our research team has found reduced thiamine concentrations in the egg-yolk, liver, and brain, as well as reduced activities of the thiamine-dependent enzymes transketolase and α-ketoglutarate dehydrogenase in the liver and brain. In these organs, there were also very high proportions of apoenzyme of these enzymes. The thiamine status of females in the field was critical for the degree of breeding failure. The many observations of advanced thiamine deficiency in adult birds strongly suggest that also varying degree of moderate thiamine deficiency occurs among the affected species.
One effect of moderate thiamine deficiency is altered behaviour. We have made plenty of observations of reduced aggressiveness and low noise level in bird colonies, as well as incomplete nest building and egg laying in nests of other species. The observed degree of thiamine deficiency suggests that the birds also suffer from weakening of the blood-brain barrier and immune suppression. This is not only an obvious threat to ecosystem sustainability. For example, infectious diseases may be more easily spread to humans by immunosuppressed birds. The thiamine deficiency may be induced either by a causative agent(s) acting directly on the affected individual, and/or by insufficient transfer of thiamine between the trophic levels in the food web.

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Periconceptioanal folic acid and folic acid containing multivitamins for the prevention of cardiovascular malformations

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The prevention of neural-tube defects with periconceptional folic acid or folic acid-containing multivitamin supplementation is accepted by the scientific community and used in the medical practice though the findings of intervention trials and observational studies showed also the efficacy of this preventive method for cardiovascular malformations, the most common structural birth defects. The aim of this presentation is to summarise the findings of previous studies regarding the prevention of cardiovascular malformations by folic acid or folic acid-containing multivitamin in early pregnancy completed by the recent results of the Hungarian population-based case-control studies in the different types of cardiovascular malformations.

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Preterm birth preventive effect of folic acid

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Recently the fetal weight promoting effect of folic acid (FA) has been stated by some medical doctors as an argument against this preventive method, because the large birthweight may associate with a higher risk for birth complications of newborns. The population-based dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities was appropriate to test the hypothesis regarding the possible fetal growth promoting and/or preterm birth reduction effect of FA supplementation during pregnancy, particularly in the third trimester. Only one type of 3 mg FA tablet was available in Hungary during the study period. The reference group included pregnant women without FA and multivitamin supplementation before conception (at least 3 months) and during the study pregnancy. Our sample included 38,151 newborns, thus represented 1.8% of Hungarian births, and 19,102 (50.0%) had mothers with FA supplementation. The mean gestational age at delivery was 39.5+2.0 and 39.2+2.1 in pregnant women with FA supplementation and in the reference group, respectively ($p < 0.0001$). The mean birth weight was 3,294+501 g in the newborns of pregnant women with FA supplements and 3,262+509 in the reference group ($p=0.82$). The rate of preterm births was 7.6% in the group of pregnant women with FA supplementation while 11.1% in the reference group, and this 3.5% difference was significant (OR with 95% CI: 0.68, 0.63-0.73). The rate of low birthweight was also lower in the FA group (5.1%) than in the reference group (6.8%) but this 1.1% difference was not significant (OR with 95% CI: 0.88, 0.62-1.14). In the next step the possible association of longer gestational age and particularly the lower rate of preterm births after FA supplementation were analyzed according to different trimesters. The longest gestational age and lowest rate of preterm birth was found after FA supplementation during the entire pregnancy, i.e. I-III trimesters (39.8 wk and 4.9%) and the II-III trimesters (39.9 wk and 3.8%), followed by the III trimester alone (39.5 wk and 7.6%). This trend resulted in 39.8 wk of mean gestational age and 4.8% rate of preterm birth in the group of III trimester together (III + II-III + I-III trimesters). Our data suggest that the third trimester is a key time window of FA supplement use for the reduction of preterm births.

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Primary prevention of congenital abnormalities with specified origin

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These studies were based on the large population-based Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980-1996 including 22,843 cases with different congenital abnormalities (CAs) and 38,151 matched controls without any CA.
Maternaldiabetes mellitus (DM) during pregnancy associates with a higher risk of CAs in their offspring. Our study was focused on pregnant women with DM-1, and a 1.5-fold higher risk of CAs was found in the offspring of these pregnant women. However, there was no higher risk of total CA in the offspring of diabetic pregnant women with folic acid supplementation. NTD and renal a/dysgenesis did not occur, and there was no higher risk of obstructive CAs of urinary tract. However, the risk of cardiovascular CAs and multiple CAs has remained higher in the subgroup of diabetic pregnant women with folic acid supplementation.
Epilepsyis one of the most frequently studied maternal diseases during pregnancy because most epilepsies had an early onset therefore epilepsy occurs in 0.3-0.6% of pregnant women and their treatment with antiepileptic drugs associates with the higher rate of CA-syndromes (e.g. fetal hydantoin/phenytoin and fetal valproate syndrome/effect) and isolated CAs. The teratogenic potential

of carbamazepine, phenobarbital, phenytoin and primidone was reduced but not eliminated by folic acid supplementation in early pregnancy.
High fever related maternal infectious diseasessuch as influenza, common cold with secondary complications and tonsillitis showed an increased risk for total CAs explained by some specific CAs if these diseases occurred in the critical period of these CAs. Our data showed that folic acid/multivitamin supplementation can reduce and/or protect against the teratogenic effects of high fever in neural-tube defect and cleft lip ± palate, but not all CAs, e.g. cannot reduce the high fever related risk of multiple CA.

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Low Hydrogen Sulfide and Chronic Kidney Disease: A Dangerous Liaison

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Hydrogen sulfide, H_2S, is a gaseous compound involved in a number of biological responses, e.g. blood pressure, vascular function, energy metabolism. In particular, H_2S is able to lower blood pressure, to protect from injury in models of ischemia-reperfusion, and to induce a hypometabolic state. In chronic kidney disease (CKD), low plasma hydrogen sulfide levels have been established in humans and in animal models. The enzymes involved in its production are cystathionine b-synthase, cystathionine g-lyase, and 3-mercaptopyruvate sulfurtransferase. The mechanisms for H_2S decrease in CKD are related to the reduced gene expression (demonstrated in uremic patient blood cells) and decreased protein levels (in tissues such as liver, kidney, brain in a chronic kidney disease rat model). It has been shown that alterations in this pathway complicate the uremic state and are linked to chronic kidney disease progression. It remains to be established if low H_2S is causally linked to CKD progression and if interventions aimed to restore the status quo ante are able to modify this picture.

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