11th International Conference on Homocysteine & One-Carbon Metabolism
14 to 18 May 2017
ICHOCM 2017

PROGRAM & ABSTRACTS
14 to 18 MAY 2017
AARHUS UNIVERSITY
AARHUS, DENMARK
WELCOME TO THE 11TH INTERNATIONAL CONFERENCE ON HOMOCYSTEINE & ONE-CARBON METABOLISM

Dear conference participants

We are delighted to welcome you to Aarhus and Denmark and we look forward to four days of fantastic scientific inspiration in this field that we are all so deeply engaged with.

The beautiful Aarhus University Campus will provide the setting for the conference where new and old friends hopefully will create a lively atmosphere that will inspire all the participants. Aarhus University is a top 100 university and holds a strong position in health sciences, being one the most ambitious and high ranking in Northern Europe. The close collaboration with the Aarhus University Hospital and both national and international institutions creates perfect conditions for research and education at a high international level.

The topic of the conferences is “Taking science to the next level – challenging paradigms and conventions”. We encourage all presenters to think about the conventions and paradigms in the expert fields, and consider whether these should be revisited and challenged. We want to give the participants the chance of learning the newest and best within our scientific field. The program holds 18 scientific sessions, 60+ oral presentations, of which approx. half were selected from the 90 submitted abstracts. Further, 50+ posters will be presented. We hope that you will enrich the conference by participating and sharing with us your latest knowledge and experiences, engaging in discussions and networking activities, and thereby contributing to a rewarding conference.

The conference will serve as a tribute to Professor Ebba Nexo. Her scientific achievements are outstanding and cover several decades of research at the frontline of our field. She will open the scientific program and she will be rewarded attention throughout the conference.

Sincerely,

Johan Frederik Håkonsen Arendt  
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Department of Clinical Biochemistry  
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Local organizing committee

Eva Greibe, Aarhus University Hospital, Denmark
Johan Frederik Håkonsen Arendt, Aarhus University Hospital, Denmark
Ebba Nexo, Aarhus University Hospital, Denmark

Conference organizing partner

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www.kongreskompagniet.dk
Professor Ebba Nexo is the guest of honor at the conference. The conference is held as a tribute to her for her life-long outstanding contribution to our scientific field.

Professor Ebba Nexo has a MD and a Dr.Med.Sci. degree. She holds a professorship in clinical biochemistry at the University of Aarhus, and she has long standing achievements within the areas of vitamins and growth factors.

Notably, she has contributed with both basic and clinical aspects related to vitamin B12 often in collaboration with international colleagues and as part of several EU-projects. Currently she is participating in two research programs, the TRIM project and the Danish-Indian IMPROVIT project.

She has served as chairman for the Danish MRC and as vice chair for the board of the Danish Research Councils. On the European level she has been a member of the steering committee for EUROHORCS and member of the advisory board of ESF.
Steven H. Zeisel, MD, PhD is Kenan Distinguished University Professor in Nutrition and Pediatrics; former Chairman, Department of Nutrition; Director Nutrition Research Institute and Director UNC Nutrition Obesity Research Center (MD, Harvard, 1975; PhD, M.I.T., 1980).

The Nutrition Research Institute focuses on using genetic, epigenetic and metabolomic methods to discover why there is individual variation in responses to, and requirements for nutrients. The UNC Nutrition Obesity Research Center is one of twelve centers of excellence in nutrition research funded by the US National Institutes of Health. Dr. Zeisel’s research focuses on dietary requirements for the nutrient choline, genetic variation as a source of individual differences in requirements for, and responses to nutrients, effects of choline and folate on stem cell proliferation and apoptosis and resulting effects on cancer and neurogenesis. His research team works with cells, mouse models, and human clinical studies. Dr. Zeisel is the author of more than 250 peer reviewed scientific papers. He is on the editorial board of the FASEB Journal and is an editor of the nutrition textbook “Present Knowledge of Nutrition, Volume 10.” Dr. Zeisel is a leader in the development of an innovative nutrition curriculum used by more than 150 medical schools.
Professor J. David Spence

J. David Spence, BA, MBA, MD, FRCP, FAHA, FCAHS
Professor of Neurology and Clinical Pharmacology,
Robarts Research Institute, Western University, London, Canada
Professor Spence has focused on prevention of stroke throughout his career. He pioneered the measurement of 2-dimensional carotid total plaque area beginning in 1990, and since 1994 has collaborated with Prof. Aaron Fenster and Dr. Grace Parraga at the Robarts Research Institute in measurement of 3D plaque volume, plaque ulceration and assessment of vulnerable plaque. He also pioneered a new approach to vascular prevention - “treating arteries instead of treating risk factors”, that has markedly reduced risk among high-risk patients with carotid stenosis. His research program focuses on measurement of atherosclerosis by ultrasound, for patient management, genetic research and for assessing effects of new therapies.

His interest in homocysteine began in 1986, with trying to understand premature atherosclerosis. He has published papers on the relation of homocysteine to carotid plaque burden, regression of carotid plaque with homocysteine lowering, was a member of the Executive Committee of the Vitamin Intervention for Stroke Prevention (VISP) trial, was PI of the Diabetic Intervention with Vitamins in Nephropathy (DIVINe) study that showed harm of B vitamins in patients with renal failure, and has been a leader in unraveling the complexity of the evidence for homocysteine lowering in stroke prevention.
Practical Information

- Invited speakers, oral and flash presenters can upload their presentation in the auditorium of the session (see program) at any time during the conference, but no later than on the morning of their presentation.
- Posters: Pins to mount your poster will be provided. Posters in Poster Session 1 can be mounted from Monday May 15 at 8:00 and should be removed no later than Tuesday May 16 at 17:00. Posters in Poster Session 2 can be mounted from Wednesday May 17 at 8:00 and should be removed no later than Thursday May 18 at 11:00. Posters from oral and flash presenters will be located separate from the other posters and do not need to be removed until Thursday May 18 at 11:00. No responsibility will be taken for posters that are not removed in due time.
- Please wear your name badge during the conference, as university students also use the Lake Auditorium and the cafeteria during the conference.
- Morning & afternoon coffee is served at the Lake Auditorium.
- Lunch is served at the cafeteria for Social & Political Science (3 minute walk, see map)
- Transportation: Coaches depart from Radisson Blu Scandinavia Hotel to the conference venue every morning at: Monday: 7:50 + 8:30; Tuesday: 8:30; Wednesday: 8:30; Thursday: 8:50. Coaches depart from the conference venue to Radisson Blu Scandinavia Hotel at 16:30 Monday and at 16:40 Tuesday+Wednesday. The walk from the hotels to the conference venue is 1-2 km.
- Welcome reception at Aarhus City Hall, Soender Allé 2, DK-8000 Aarhus C is on Monday 15th of May from 17:30-19:00. Direct transportation is not provided, but coaches leave for Radisson Blu Scandinavia Hotel at 16:30 and the City Hall is near the hotel. A 1-hour guided walk in Aarhus city center is offered free of charge after the Welcome reception, starting right outside the City Hall.
- Conference Dinner at Centralvaerkstedet, Vaerkmestergade 7-9, DK-8000 Aarhus C is on Wednesday 17th of May from 19:00. Coaches depart from “Women’s museum” near CabInn (18:20), Radisson Blu Scandinavia Hotel (18:30), and the Mayor Hotel (18.40). The walk from the hotels to the dinner venue is less than 1 km
- All sessions take place in the Lake Auditorium. Plenary sessions: Per Kirkeby Auditorium. Parallel sessions: Merete Barker Auditorium & Jeppe Vontillius Auditorium. Poster sessions: Hallways
- Free WiFi is available. To access free WIFI on Campus you should: 1) Connect to AU Guest; 2) Open a browser; 3) Login via the web portal that pops up automatically. You can login via Facebook, Google, LinkedIn, SMS (only Danish phone numbers) and Microsoft. Guest access is for the internet only and the access is limited to the web (http and https).
- No smoking allowed inside the Lake Auditorium
ARoS Art Museum
Radisson Blu Scandinavia Hotel
Aarhus City Hall
Aarhus Bus Station
The Mayor Hotel
Aarhus Railway Station
Conference Dinner site Centralvaerkstedet
Program for the 11th International Conference on Homocysteine & One-Carbon Metabolism 2017

Sunday 14 May 2017
14:00-16:30 Registration and free entrance at the Aros Arts Museum, Aros Allé 2, DK-8000 Aarhus C

Monday 15 May 2017
08:00-09:00 Registration at the Lake Auditorium Reception

Session 1: Challenging paradigms and conventions
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Ralph Green and Jacob Selhub

09:00-09:20 Welcome by Johan F. H. Arendt and Eva Greibe, Organizers, Denmark

09:20-10:05 Black holes in the universe of vitamin B12
Speaker: Ebba Nexo, Guest of honor, Aarhus, Denmark – Abstract O1

10:05-10:30 Elevated vitamin B12 levels – overlooked or overrated?
Speaker: Johan F. H. Arendt, Aarhus, Denmark – Abstract O2

10:30-10:50 Morning coffee break – Lake Auditorium Hallways/William Scharff Auditorium

Session 2: B-vitamins and genetics – what can we learn from rare genetic disorders?
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Henk Blom and Jean-Louis Gueant

10:50-11:15 From the clinic to the mouse room - Lessons learned in the regulation of folate transport
Speaker: Richard Finnell, Texas, USA – Abstract O3

11:15-11:40 Surprises from the study of patients with inherited disorders of cobalamin metabolism
Speaker: David Rosenblatt, Montreal, Canada – Abstract O4

Session 3: Consensus guidelines for inherited disorders
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by David Rosenblatt and Brian Fowler

11:40-12:05 European network and registry for homocystinurias and methylation defects (E-HOD)
Speaker: Henk Blom, Freiburg, Germany – Abstract O5

12:05 –12:30 EHOD in practice – a South American physician’s perspective on the "placebo effect" of rare disease registries
Speaker: Ida V. D. Schwartz, Porto Alegre, Brazil – Abstract O6
SESSION 4: Defining diagnoses – are biochemical cut-offs ancient history?
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Ebba Nexo and Sally Stabler

13:40-14:05  Combined indicator of B12 deficiency (cB12). Optimized sequential application of individual biomarkers and their combinations
Speaker: Sergey Fedosov, Aarhus, Denmark – Abstract O7

14:05-14:30  Assay of cobalamin analogues in blood and animal tissues with potential contribution to cobalamin values
Speaker: Sally Stabler, Colorado, USA – Abstract O8

14:30-14:55  One-carbon metabolism and vitamin nutritional status – metabolomic assessment in health and disease states
Speaker: Jesse Gregory, Florida, USA – Abstract O9

14:55-15:00  Vitamin B12 and folate status of older Irish adults: findings from TILDA
Speaker: Eamon Laird, Dublin, Ireland – Abstract 10

15:00-15:30  Afternoon coffee break – Lake Auditorium Hallways/William Scharff Auditorium

SESSION 5: B vitamins and life-style factors
Parallel session. Conference room: Lake Auditorium Jeppe Vontilius
Chaired by Anne Molloy and Shantanu Sengupta

15:30-15:55  One-carbon metabolism and fetal programming
Speaker: Chittaranjan Yajnik, Pune, India – Abstract O11

15:55-16:10  Markers of folate and vitamin B12 status are associated with insulin resistance and metabolic syndrome severity in morbidly obese patients
Speaker: Jean-Louis Gueant, Nancy, France – Abstract O12

16:10-16:15  Profiling of folates and related sulfur amino acids in SHR rat model of metabolic syndrome
Speaker: Victor Kozich, Prague, Czech Republic – Abstract O13

16:15-16:20  Genetic variation in one-carbon metabolism, prenatal air pollution exposure, and cardiovascular phenotypes in children
Speaker: Caitlin Howe, California, USA – Abstract O14

16:20-16:25  The effect of folic acid supplementation on insulin sensitivity and type 2 diabetes – A meta-analysis of randomized controlled trials
Speaker: Mads Lind, Copenhagen, Denmark – Abstract O15
SESSION 6: Animal models for B vitamin-related pathologies
Parallel session. Conference room: Lake Auditorium Merete Barker
Chaired by Richard Finnell and Joshua Miller

15:30-15:55  Role of folate receptor antibodies in neuro-developmental disorders
Speaker: Edward Quadros, New York, USA – Abstract O16

15:55-16:10  Successful generation of a neuronal conditional Lmbrd1 knockout mouse
Speaker: Frank Rutsch, Muenster, Germany – Abstract O17

16:10-16:15  Engineering and characterization of an enzyme replacement therapy for classical homocystinuria
Speaker: Tomas Majtan, Colorado, USA – Abstract O18

16:15-16:20  Transcriptional repression of male-specific MUP20 expression in female mice is relieved by the inactivation of cystathionine-β synthase gene
Speaker: Hieronim Jakubowski, New Jersey, USA – Abstract O19

16:20-16:25  One-Carbon metabolism and neural tube closure defects
Speaker: Nicholas Greene, London, UK – Abstract O20

SOCIAL EVENT:
17:30-19:00  Welcome reception
City Hall, Soender Allé 2, DK-8000 Aarhus C

TUESDAY 16 May 2017
SESSION 7: Nutrition during pregnancy and infancy – pathology and physiology
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Eva Greibe and Alex Brito

09:00-09:25  B vitamin status in non-pregnant, pregnant and lactating mothers and their infants
Speaker: Anne-Lise Bjorke-Monsen, Bergen, Norway – Abstract O21

09:25-09:50  Parental one carbon metabolism, pregnancy outcome and child development and health
Speaker: Michelle Murphy, Reus, Spain – Abstract O22

09:50-10:05  Utility of newborn screening markers to diagnose newborns at risk of vitamin B-12 deficiency second to maternal vitamin B-12 deficiency
Speaker: Theresa Schroder, Vancouver, Canada – Abstract O23

10:05-10:10  Breast milk levels of one-carbon metabolites and infant postnatal growth
Speaker: Carles Lerin, Barcelona, Spain – Abstract O24

10:10-10:15  Higher cord blood unmetabolized folic acid (PGA) levels are associated with the development of food allergy in children
Speaker: Jacob Selhub, Virginia, USA – Abstract O25

10:15-10:30  The effect of folate on vitamin B12 depletion-induced inhibition of nuclear thymidylate biosynthesis and neural tube defects
SESSION 8: Molecular and cellular mechanisms of B vitamins
Parallel session. Conference room: Lake Auditorium Jeppe Vontilius
Chaired by Viktor Kozich and Sergey Fedosov

11:00-11:25 Cellular stress is a key actor of a common scenario, which participates to the high variability of neurological and visceral manifestations of cobalamin disorders
Speaker: Jean-Louis Gueant, Nancy, France – Abstract O27

11:25-11:50 Molecular characterization of the cblC disease reveals new pathways in pathogenesis
Speaker: Luciana Hannibal, Freiburg, Germany – Abstract O28

11:50-12:05 Methionine synthase and methionine synthase reductase interact with MMACHC and with MMADHC
Speaker: David Coelho, Nancy, France – Abstract O29

12:05-12:10 Arsenic Targets Folate-dependent de novo Thymidylate Synthesis in the Nucleus Leading to Neural Tube Defects
Speaker: Patrick Stover, New York, USA – Abstract O30

SESSION 9: Nutrition and disease
Parallel session. Conference room: Lake Auditorium Merete Barker
Chaired by Sally Stabler and Barry Shane

11:00-11:45 New perspectives on vitamin B-12 deficiency in low income populations; assessment and prevalence
Speakers: Lindsey Allen and Alex Brito, California, USA – Abstract O32

11:45-12:00 Cow milk, buffalo milk or vitamin pills for improving vitamin B12 status: A four week prospective study
Speaker: Sadanand Naik, Pune, India – Abstract O33

12:00-12:15 Genetic epidemiology of static and functional biomarkers of vitamin B12 status in older adults
Speaker: Kourosh Ahmadi, Surrey, UK – Abstract O34

12:15-12:20 Vitamin B12 deficiency affects distinct pathways in males and females
Speaker: Swati Varshney, New Delhi, India – Abstract O35

12:20-13:30 Dine with a Senior Researcher – Cafeteria for Social and Political Sciences
Chair: Ebba Nexo, Aarhus, Denmark
SESSION 10: Poster session 1
Lake Auditorium Hallways
Chaired by Michelle Murphy, Alex Brito, Ebba Nexo and Richard Finnell

13:30-14:30  Poster session 1

14:30-15:00  Afternoon coffee break – Lake Auditorium Hallways/William Scharff Auditorium

SESSION 11: Cognition, dementia, and B vitamins – animal models, trials and epidemiology
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Johan F. H. Arendt and Jean-Louis Gueant

15:00-15:25  How does the brain mediate the effects of low vitamin B12 and raised homocysteine on cognition?
Speaker: David Smith, Oxford, UK - Abstract O36

15:25-15:50  Homocysteine and the Brain-Body divide
Speaker: Aron Troen, Jerusalem, Israel - Abstract O37

15:50-16:05  Behavioral alterations associated with vitamin B12 deficiency in the TCblR/CD320 KO mouse
Speaker: Jeffrey Sequiera, New York, USA - Abstract O38

16:05-16:20  Vitamin B12, folate, and sulfur amino-acids as risk factors for dementia and cognitive decline: a longitudinal population based study
Speaker: Babak Hooshmand, Stockholm, Sweden – Abstract O39

16:20-16:25  Folate receptor alpha autoimmunity in low-functioning autism and response to folinic acid treatment
Speaker: Vincent Ramaekers, Liége, Belgium – Abstract O40

16:25-16:30  Plasma and cerebrospinal fluid biomarkers of the methylation cycle in cognitively normal and mild dementia of the Alzheimer type
Speaker: Teodoro Bottiglieri, Texas, USA – Abstract O41

WEDNESDAY 17 May 2017
SESSION 12: B vitamins and cancer
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by David Smith and Ottar Nygård

09:00-09:40  Challenges to evaluating evidence of risks from high intakes of folic acid
Speaker: Abee L. Boyles, North Carolina, USA – Abstract O42

09:40-09:55  Impaired functional vitamin B6 status is associated with increased risk of lung cancer in the Lung Cancer Cohort Consortium (LC3)
Speaker: Despoina Theofylaktopoulou, Bergen, Norway – Abstract O43
SESSION 13: Bioavailability of B vitamins
Parallel session. Conference room: Lake Auditorium Jeppe Vontilius
Chaired by Ralph Green and Michelle Murphy

10:55-11:20 Principle for studying the potency of the different vitamin D active compounds – usable in the vitamin B community?
Speaker: Jette Jakobsen, Copenhagen, Denmark – Abstract O46

11:20-11:45 B12 in milk, its protein complexes and bioavailability
Speaker: Sergey Fedosov, Aarhus, Denmark – Abstract O47

11:45-12:00 Development and validation of the concept of personalized folate supplementation to achieve protective RBC-folate concentrations in young women
Speaker: Klaus Pietrzik, Bonn, Germany – Abstract O48

SESSION 14: Betaine and Choline – the link to B vitamins and homocysteine
Parallel session. Conference room: Lake Auditorium Merete Barker
Chaired by Steven Zeisel and Viktor Kozich

10:55-11:40 How do perturbations in choline metabolism cause altered gene expression?
Speaker: Steven Zeisel, North Carolina, USA – Abstract O51

11:40-11:55 Results from a folic acid and creatine supplementation trial in Bangladesh: Differences in treatment effect on arsenic methylation, blood arsenic, and homocysteine by baseline choline and betaine status
Speaker: Anne Bozack, New York, USA – Abstract O52

11:55-12:10 Serum betaine and total vitamin B-12, but not folate, concentrations are negative predictors of total homocysteine concentration in Canadian pregnant women of South Asian and Caucasian ethnicity
Speaker: Maria Fernanda Mujica-Coopman, Vancouver, Canada – Abstract O53
12:10-12:15  A Systematic Review and Meta-analysis on the efficacy of Betaine in the treatment of Cystathione B-synthase deficient patients who are non-responsive to pyridoxine
Speaker: Alicia Dorneles Dornelles, Porte Alegre, Brazil – Abstract O54

12:15-12:20  The Effects of Betaine on Brain and Liver in Methionine & Choline Deficient Rats
Speaker: Nur Abu Ahmad, Jerusalem, Israel – Abstract O55

12:20-13:30  Lunch buffet – Cafeteria for Social and Political Sciences

SESSION 15: Poster session 2
Lake Auditorium Hallways
Chaired by Joshua Miller, Luciana Hannibal, Lindsay Allen and Barry Shane

13:30-14:30  Poster session 2

14:30-15:00  Afternoon coffee break - Lake Auditorium Hallways/William Scharff Auditorium

SESSION 16: B vitamins and treatment
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Johan F. H. Arendt and Anne Molloy

15:00-15:25  Cyano-B12 versus hydroxo-B12: New insights on transport and accumulation
Speaker: Eva Greibe, Aarhus, Denmark - Abstract O56

15:25-15:50  Bacterial folates provide an exogeneous signal for C. elegans germline stem cell proliferation
Speaker: Jacob Selhub, Massachusetts, USA - Abstract O57

15:50-16:05  Vitamin B12 status and absorption before and after bariatric surgery: A prospective study
Speaker: Linda Kornerup, Aarhus, Denmark – Abstract O58

16:05-16:20  Paraoxonase 1 protects against homocysteine-thiolactone accumulation in humans
Speaker: Joanna Perla-Kajan, Poznan, Poland – Abstract O59

16:20-16:25  The role of homocysteine and B vitamins in telomere length: results from the cross-sectional and interventional trials
Speaker: Irene Pusceddu, Saarland, Germany – Abstract O60

16:25-16:30  Development of an Enzyme Therapeutic for Classical Homocystinuria
Speaker: Matthew Bonem, Texas, USA – Abstract O61

SOCIAL EVENT:
19:00-  Dinner party
Centralvaerkstedet, Vaerkmestergade 7-9, DK-8000 Aarhus C
(coaches will be arranged)
Festive speaker: Ralph Green, California, USA
THURSDAY 18 May 2017
SESSION 17: B vitamins, homocysteine and cardiovascular disease
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by Jacob Selhub and David Smith

09:30-09:55 Lowering homocysteine levels to prevent stroke: unraveling the complexity of the evidence
Speaker: J. David Spence, London, Canada – Abstract O62

09:55-10:20 Plasma Homocysteine, B vitamin treatment and cardiovascular disease: still relevant to study?
Speaker: Ottar Nygård, Bergen, Norway – Abstract O63

10:20-10:35 Studies on the influence of folic acid, riboflavin, and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism on nitric oxide production and blood pressure
Speaker: Joshua Miller, New Jersey, USA – Abstract O64

10:35-10:50 Neopterin as an effect modifier of the cardiovascular risk associated with total homocysteine – a two-cohort study of patients with coronary heart disease
Speakers: Espen Bjørnestad and Robert Borsholm, Bergen, Norway – Abstract O65

10:50-11:15 Morning coffee break – Lake Auditorium Hallways/William Scharff Auditorium

SESSION 18: Future for the conference and research society
Plenary session. Conference room: Lake Auditorium Per Kirkeby
Chaired by David Rosenblatt and Henk Blom

11:15-11:40 The International Conference on Homocysteine and One-Carbon Metabolism: Quo Vadis
Speaker: Brian Fowler, Basel, Switzerland - Abstract O66

11:40-12:05 The Pernicious Anaemia Society
Speaker: Martyn Hooper, Founder & Chair of PAS – Abstract O67

12:05-12:30 Discussion

12:30-13:00 Closing remarks and announcing oral and poster prize winners
Johan F. H. Arendt, Eva Greibe, Ebba Nexo

13:00 - Lunch boxes for departure – Lake Auditorium Hallways
Abstracts

Oral presentations

SESSION 1: Challenging paradigms and conventions

O1 Ebba Nexo Black Holes in the Universe of Vitamin B12

Ebba Nexo1,2.
1Department of Clinical Biochemistry, Aarhus University Hospital; 2Department of Clinical Medicine, Aarhus University, Aarhus, Denmark.

Once the vitamin curing pernicious anemia was identified, crystalized and introduced in the clinic many considered this as a remarkably medical fairy tale with a happy ending and no more to be said.

They were wrong. Since the middle of the 20th century the number of publications on vitamin B12 (B12) has increased and has currently leveled out at around 900 per year. Many both basic and clinical aspects have been clarified only to enlighten new and old problems yet to be addressed. This lecture will focus on trafficking. We know that various forms of B12 are absorbed alike, but knowledge on body distribution and choice of B12 form and dose for prevention or treatment of an impaired B12 status is still up for debate.

Most circulating B12 is bound to haptocorrin, present also in most extracellular fluids. Haptocorrin binds both active and inactive B12, but its function is yet to be unraveled.

The minor part of circulating B12 bound to transcobalamin is recognized by CD320, a receptor present on many but not all cells, and thus we still need to identify additional receptors.

Once within the cell B12 needs to get out again, to the circulation, to the fetus, the milk and the enterohepatic circulation. So far the molecular mechanisms and the cellular turnaround time of the vitamin remains to be clarified, as does the limitations and benefits of employing the B12 transport system for delivery of exogenous substances.

Taken together we need more knowledge on trafficking of B12 in order to optimize strategies for prevention and treatment of an impaired B12 status and in order to employ the B12 transport system as carrier for drugs and diagnostic substances.

O2 Johan F. H. Elevated vitamin B12 levels – overlooked or overrated?

Johan F. H. Arendt1,2.
1Department of Clinical Epidemiology, Aarhus University Hospital; 2Department of Clinical Biochemistry, Aarhus University Hospital.

Recent scientific work has confirmed what was first shown more than 70 years ago: that high plasma levels of vitamin B12 (B12) are associated with severe disease, most importantly the short-term risk of cancer. The interpretation of this association is that cancer give rise to high B12 levels; not that neither high B12 intake nor high-dose medication increases the risk of cancer. The most well described disease in this context is chronic myeloid leukemia. Here, proliferating malignant leucocytes produces the B12-binding protein, haptocorrin, giving rise to high B12 levels. However, this rare disease cannot explain why a significant proportion of patients have high B12 levels and there is little knowledge on the underlying alterations in B12 metabolism in most of the diseases that are associated with elevated B12. Moreover, earlier studies were based on patient populations and are likely biased, making it difficult to assess whether elevated B12 is associated with disease in the general population. In general, there are more questions than answers regarding the clinical implications of high B12 levels.

The need to better understand the clinical implications and the underlying pathophysiological mechanisms will be the focus of this presentation, and suggestions will be put forward to fill the knowledge gaps on elevated B12 levels using already existing data sources. The clinical implications are still unresolved, but the risk of several severe diseases in persons with elevated B12 levels may have been overlooked in the past.
SESSION 2: B-vitamins and genetics – what can we learn from rare genetic disorders?

O3  Richard H. Finnell From the Clinic to the Mouse Room - Lessons Learned in the Regulation of Folate Transport


1Dell Pediatric Research Institute, Department of Pediatrics, The University of Texas at Austin Dell Medical School, Austin, Texas, USA; 2Department of Pediatrics and Pediatric Neurology, University Medical Center Goettingen, Germany.

Cerebral folate deficiency (CFD) syndrome is characterized by very low concentration of 5-methyltetrahydrofolate (5-MTHF) in cerebrospinal fluid, while folate levels in plasma and red blood cells are within normal range. CFD patients present with symptoms including: developmental delay, ataxia, dyskinesias, spasticity, speech difficulties and epilepsy. Previously, mutations in several folate pathway genes, including hFRα (folate receptor alpha), DHFR (dihydrofolate reductase), and PCFT (proton coupled folate transporter) have been identified in CFD patients.

To identify causal mutations for CFD, we performed whole exome sequencing (WES) on DNA samples collected from a CFD patient, her healthy sibling, and her biological parents. A de novo mutation in human Capicua gene (CIC), c.1057C>T (p.R353X), was identified in the patient and confirmed using Sanger sequencing. A second CFD patient was confirmed as a compound heterozygote of two splice site variants of CIC gene, IVS1360+32G>AG and IVS3796-15C>CT. A missense mutation predicted to be damaging, c.1738G>GT (p.G580GC) was identified in a 3rd CFD patient. We have now evaluated close to 70 individuals diagnosed with CFD, performing both genomic and in vitro functional analyses. Further clinical work-up of all patients failed to reveal any abnormalities in brain imaging but indicated disturbed cerebral folate transport.

The CIC protein is a HMG-box transcriptional repressor. The DNA binding domain located at amino acid residues 200-268 binds the octomer sequence T(G/C)AATG(A/G)A. The mutation in CFD patient #`, p.R353X, caused the protein to degrade through nonsense-mediated mRNA decay (NMD); therefore, less CIC protein could be detected in the patient’s fibroblast and iPS cells. CIC target binding octomer sequence has been found in the promoter regions of folate transport genes FOLR1, PCFT, RFC1, and DHFR, which is involved in folate metabolism. In the patient’s iPS cell, the p.R353X mutation down regulated FOLR1, PCFT and RFC1 gene expression compared with H9 stem cells and an iPS cell line from an individual with wildtype CIC. ChIP assays confirmed that CIC bound to the FOLR1, PCFT and RFC1 promoter in vitro. In dual-luciferase assay, the CIC protein repressed FOLR1 promoter transcription. Further, we provide evidence for direct transcriptional regulation of the FOLR1 gene by CIC. Since, hFRα has been shown to mediate the folate transport across the choroid plexus (Grapp et al., Nat Commun. 2013;4:2123) loss of CIC function could result in CFD. The lack of cerebral folate explains the patient symptoms such as autism, developmental delay and epilepsy, all characteristic of CFD syndrome.

Cic null and conditional knockout mice were created and a systematic behavioral and molecular analysis demonstrated that CIC functions at the hypothalamus and medial amygdala to modulate social interactions. CIC is also active in the forebrain, where loss of CIC results in impaired learning and memory. Histological and molecular analyses demonstrated reduced thickness of upper cortical layers in the developing forebrain of Cic conditional knockout mice, abnormal postnatal post-mitotic maturation, and maintenance of upper layer cortical neurons. These brain regional Cic deficient mice showed similar phenotypes as did the CFD proband, including ASD-like behaviors and development delay. Thus, a fortuitous clinical interaction led to intensive mouse genetic studies that supported a heretofore understudied regulatory mechanism of folate transport.

These studies were funded in part by NIH grants HD067244 and HD081216.

O4  David S. Rosenblatt Surprises from the study of patients with inherited disorders of cobalamin metabolism

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The study of cultured cells from patients referred because of elevations of homocysteine,
methylmalonic acid, or both in blood and/or urine has allowed the characterization of genes responsible for the inherited disorders of cobalamin transport and metabolism. Recently there have been surprising discoveries implicating regulatory mechanisms in disease. One of the biggest is that some patients who have a cellular phenotype identical to that of patients with the cblC disorder actually have mutations in genes leading to down regulation of MMACHC expression. What is particularly surprising is that cells from these patients fail to correct those from cblC patients in somatic cell complementation analysis. One set of patients has mutations in the HCFC1 gene, which is on the X chromosome. For this reason, their disease has been called “cblX” even though it does not represent a true new complementation class. In addition, a single patient is known with a homozygous mutation in the THAP11 gene; this patient has a similar clinical and cellular phenotype to the cblC patients. We have recently described another patient in whose cells there is down regulation of MMACHC, but in whom the cellular phenotype differs from that of the cblC disorder. Transcobalamin (TC)-cobalamin is taken up normally but does not dissociate within cells, suggesting a block in cobalamin uptake at an earlier step than that affected by the cblC disorder, prior to the dissociation of cobalamin from TC in lysosomes. Compound heterozygous mutations were identified in ZNF143, which codes for a transcription factor known to form a complex with HCFC1 and THAP11. We suggested that the defect in cobalamin metabolism in this patient is the result of altered expression of an unidentified gene specifically regulated by ZNF143, independent of HCFC1 and THAP11.

SESSION 3: Consensus guidelines for inherited disorders

O5 Henk J. Blom European network and registry for homocystinurias and methylation defects (E-HOD)

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Background: Patients with homocystinurias (HCU), methylation defects (MD) and folate defects (FD) have an enormous need for improved medical awareness, optimization of the diagnostic process and therapy, and improved networking between healthcare professionals and patients.

Methods: An initiative named "European network and registry for homocystinurias and methylation defects (E-HOD)" was funded by the European Commission DG Sanco and started 15/02/2013. One concept E-HOD follows is that registries should be built around groups of diseases and not a single disease allowing evaluation of the phenotypic spectrum, efficacy of diagnostic and therapeutic strategies disease by disease and across single diseases. The registry has been established using a flexible IT solution that will enable to extend disease clustering in the future. In addition, available knowledge should be exploited via using existing registry technology and networks. The E-HOD consortium has three major activities: 1/ collecting longitudinal data into a registry; 2/ developing evidence-based consensus diagnostic and clinical care protocols; 3/ evaluating different newborn screening programmes for HCU and producing position papers.

Results: The E-HOD consortium consists of more than 85 partners. Many of them are from Europe but we are expanding fast world-wide, linking healthcare professionals, patients’ representatives and industry. The registry contains already more than 630 patients with HCU, MD or FD (see https://www.ehod-registry.org/). One guideline on newborn screening has been published last year and three clinical care guidelines this year. Three different patient information leaflets are translated in eight different languages. For more information see our website http://www.e-hod.org/

Conclusion: We are improving access to diagnosis and optimal care for patients with HCU, MD and FD not only within Europe but more and more world-wide. The challenge over the next years is to make E-HOD sustainable, its transition to an international consortium and exploit the registry for a better understanding of HCU, MD and FD.
EHOD in practice – a South American physician’s perspective on the “placebo effect” of rare disease registries

Ida Vanessa Doederlein Schwartz1-4.

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Registries about rare disorders are important tools for the understanding of the natural history of those diseases and for evaluation of therapy and the development of new treatments strategies. They are usually built as research projects and coordinated by professionals who are mainly based in the most developed countries. The immediate impact for patients, families, health professionals, and communities are usually not the main focus, although these registries (at least in theory) should be multicentric, multinational, and multicultural. Many efforts were made to implement EHOD in South American countries, being successful so far mainly in Brazil. In this presentation, I will give my perspective about the challenges of South American researchers and health care providers to contribute to an European Registry, and about the benefits for physicians and patients/families of such interdisciplinary approach. Registries like EHOD, by enabling interaction among several actors, create a placebo effect via the improvement of awareness about homocystinurias. It results in increased numbers of diagnosed cases, the use of the best therapeutic strategies, and the improvement of patient’s quality of life. In the case of South America, for instance, we were able to identify a potential genetic ‘isolate’ of classical homocystinuria in Colombia. In addition, educational booklets/electronic media were provided for patients/families/health professionals in Spanish and Portuguese. Meetings between patients/families and health professionals have also been organised.

SESSION 4: Defining diagnoses – are biochemical cut-offs ancient history?

Combined indicator of B12 deficiency (cB12). Optimized sequential application of individual biomarkers and their combinations

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Introduction: Diagnosis of B12 deficiency involves measurement of up to four biomarkers: total B12, holo-transcobalamin (holoTC) methylmalonic acid (MMA) and total homocysteine (tHcy). Conflicting results between the markers are smoothed if using the combined indicator of B12 deficiency, where cB12=0 corresponds to the adequate status.

Aim: We graded 4cB12 or 3cB12 (four or three markers used) in clinically characterized cases of low B12 or pernicious anemia (PA), targeting at the improved algorithms of sequential selection.

Methods: We included young Irish students (n=287); elderly Chileans, without selection (n=294), or with low B12 status (n=51); PA-patients from different studies (n=255) with B12, MMA, tHcy measured; and a large cohort from 6 countries with varying status (n=5211). Cumulative distribution function (CDF) and receiver operating characteristic (ROC) charts were used.

Results: Elderly Chileans with low 4cB12≈-1±0.5 were generally asymptomatic (10% with mild B12 or pernicious anemia (PA), targeting at the improved algorithms of sequential selection.

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Results: Elderly Chileans with low 4cB12≈-1±0.5 were generally asymptomatic (10% with mild anemia, 2% with macrocytosis) but had lower nerve conductivity. The PA-cohort was subdivided based on neurological symptoms (“0” – absent, “1+2” – light/moderate, “3+4” – advanced/severe) and plotted as CDF of 3cB12≈-1±0.5. The scale of 3cB12 showed: >-0.5 no cases of PA; -1±0.5 mostly “0”-neurology; -2±0.5 mostly “0” and “1+2”-disorders; ≤-3±0.5 accumulation of “3+4”-symptoms. ROC-charts of PA-patients vs. the generally adequate cohorts (e.g. PA vs Chilean, Figure B) sorted the markers according to their separation strength (3cB12>MMA>tHcy>B12). Sequential selection was targeted to exclude low cB12 from the large cohort. An example for B12, MMA sequence showed that 2cB12>0.5 at step-2 selected n=2525 of “adequate” including n=4 of clearly false adequate (4cB12≤-1), while MMA≤0.39μM selected n=2524 with n=13 (false).

Conclusions: The analysis suggests gradation of cB12: -1, generally asymptomatic; -2, risk of anemia
and mild neurological disorders; -3 and less, increasing risk of severe disorders. The combined indicator provides a better separation of “adequate” versus “deficient”.

**O8**  
**Sally P. Stabler**  
**Assay of Cobalamin Analouges in Blood and Animal Tissues with Potential Contribution to Cobalamin Values**

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**Introduction:** The sensitivity and especially the specificity of low cobalamin (Cbl) values are disappointing as compared to metabolic markers of deficiency such as methylmalonic acid and/or homocysteine or a defined clinical response. Human feces contain at least 8 different corrinoids including [5-OHBZA] which significantly binds to both intrinsic factor(IF) and transcobalamin (TC).

**Aim:** We assayed serum, white blood cells and many animal tissues in order to determine the types of Cbl analogues present, which in the case of those with benzimidazole-type bases could potentially bind to intrinsic factor (IF) and transcobalamin (TC) as well as haptocorrin (HC) and thus be measured as total Cbl and/or as Holo-TC.

**Methods:** Cbl and its naturally occurring analogues were measured by stable isotope dilution LC/MS after R-protein sepharose purification as previously described, AJCN (2008) 87:1324-35.

**Results:** Human serum was found to contain cobinamide (Cbi), [2-MeAde]CN-Cba (MeAde), [Ade]CN- Cba (Ade) and Cbl. Human granulocytes prepared to remain quiescent contained MeAde, Cbl and MeSAde but no Cbi. In contrast, rabbit buffy coat, red blood cells and plasma contained Cbi in addition to Cbl, MeAde and Ade. Kidney from 5 animal species had the highest concentrations of the same analogues, followed by liver. Benzimidazole containing analogues were not found in tissues or plasma. Rabbits were treated with oral [BZA]CN-Cba and had widespread dissemination of BZA to liver, kidney, heart and spleen with BZA still detectable in plasma at 96 hours post dose. BZA was rapidly excreted in the feces along with increased excretion of Cbi, 10-fold.

**Discussion:** Human serum and granulocytes contained Cbl analogues with adenine and methyl-adenine bases and serum had cobinamide (without a base). No BZA type analogues were found in body fluids or tissues. BZA is widely distributed in organs after oral dosing. The source of these analogues in blood and tissues is not known.

**O9**  
**Jesse F. Gregory**  
**One-carbon metabolism and vitamin nutritional status – metabolomic assessment in health and disease states**

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The growing field of metabolomics allows the evaluation of patterns of targeted metabolites and also the untargeted constituents in biological samples. These techniques are well suited for determining the metabolic effects of nutritional conditions and identification of metabolites that be useful as new biomarkers. Such approaches yield better insight into effects of nutrient insufficiency, may aid in nutritional status assessment, and clarify nutritional, metabolic and disease relationships. This presentation will address two examples of metabolomic analysis.

In a study of healthy young women, groups were classified as low plasma PLP (≤30 nmol/L),
adequate B6 status (31-99 nmol/L), or supplemented (PLP ≥100 nmol/L) and evaluated using targeted LCMS metabolomic analysis of one-carbon and tryptophan metabolites and global 1H-NMR analysis. Metabolite patterns distinguished among low, medium and high levels of vitamin B6 status. Major discriminating metabolites included alanine, threonine, lactate, glutathione, glucose and tryptophan catabolites. The results also indicated that 30 nmol/L is a more appropriate cutoff than the current 20 nmol/L as a criterion of vitamin B6 deficiency. We also conducted metabolomic analysis in a study assessing cardiovascular risk as a function of low versus high plasma cystathionine groups in the WECAC study. Elevated cystathionine has been shown to be associated with increased risk of AMI among patients with CHD. Results of targeted metabolomic analysis, LCMS and NMR approaches showed metabolite patterns associated with high-cystathionine. Major discriminating variables in the high cystathionine group included higher plasma glucose, lanthionine (an H2S biomarker), pyridoxic acid ratio, methylmalonic acid, methionine, choline, tryptophan, proline, creatinine, tryptophan catabolites, TMAO, and slightly higher BMI. This group also showed lower metabolites including glutathione, uridine and altered pattern of acylcarnitines, along with lower glomerular filtration rate. PLP in the high cystathionine group also tended to be lower (60.1 ± 56.1 versus 39.0 ± 20.9 μmol/L, P=0.03).

Conflicts of interest: none

O10 Eamon Laird Vitamin B12 and folate status of older Irish adults: findings from TILDA.

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Low vitamin B12 (cobalamin) status is a common finding among the elderly (1). The consequences can include megaloblastic anaemia and demyelinating neurological disease (2). Conversely, high folate status is also a major concern as it may have unintended negative effects on health (3). To-date, no large scale representative population studies have examined the status of these micronutrients in older Irish adults who are subject to a voluntary, but liberal food fortification policy.

The Irish Longitudinal Study on Ageing (TILDA) is a nationally representative sample of >8,000 older adults (≥50 yrs), aimed at investigating the health, social and economic status components of successful ageing. Blood samples were provided from participants in Wave 1 (2009-2011) and analysed for concentrations of total cobalamin (n 5,194) and plasma folate (n 5,326). Deficient vitamin B12 status was defined as <148, insufficient as 148-185 and adequate as >185 pmol/L (4). Folate status was defined as <6.8 deficient and >45 nmol/L as high folate status. Overall, 10.7% of the population sample had either deficient or inadequate vitamin B12 status. Mean concentrations were significantly higher in females (336.5 pmol/L) compared with males (314.2 pmol/L) (P<0.0001) and higher in the youngest (50-59 yrs) vs the oldest old (>80 yrs) (331.8 vs 310.7 pmol/L). For folate, 3% of the cohort were deficient while 9% had high folate status. Mean folate concentrations were significantly higher in males (19.5 nmol/L) compared with females (17.9 nmol/L) (P<0.0001). Concentrations of folate were significantly lower in the oldest old (16.8 nmol/L) compared with the youngest (18.3 nmol/L). These preliminary findings provide new data on the micronutrient status of older Irish adults and will be useful for informing public healthcare policy. The addition of these biomarkers to the TILDA dataset will allow for future exploration of the relationships between health, nutrition and healthy ageing.

Acknowledgement of Funding: Funding to conduct this work was provided by the Irish Department of Agriculture, Food and the Marine through the grant 13F492 the Nutritional Biomarker Database Enhancement Initiative of (“BIO-TILDA” 2013-2015). Original funding for the TILDA study was provided by Atlantic Philanthropies, Irish Life plc. and the Irish Government.

SESSION 5: B vitamins and life-style factors

O11 Chittaranjan S. Yajnik
King Edward Memorial Hospital and Research Centre, Pune, India.
Fetal programming refers to a permanent structural or functional change during development which influences health and disease susceptibility for the rest of life. It can be influenced by maternal nutrition, metabolism, stress, pollutants and other exposures. Animal and human work has highlighted the influence of maternal 1-C metabolism in fetal programming, methyl donors in diet being especially important. Offspring of Agouti mice fed a diet rich in methyl donors (vitamin B12, folic acid, choline, and betaine) had an altered coat colour and reduced susceptibility to obesity. This was linked to differential methylation of the promoter of the Agouti gene.

Human evidence has pointed towards the importance of maternal folate, vitamin B12 and 1-C metabolism in fetal growth and development (small for gestational age, prematurity, adipose body composition, neural tube defects, neurocognitive functions including autistic spectrum disorders, cardiac autonomic imbalance), altered neuro-endocrine response to stress, and increased risk of diabetes (adiposity and insulin resistance). Research in India has made important contributions. Indians as a group are vegetarians and have a substantial prevalence of vitamin B12 deficiency and hyperhomocysteinemia. Folate status is usually good, and national nutritional programs provide folic acid with iron but not B12. Together these factors have contributed to an imbalance between B12 and folate nutrition which seems to be associated with various adverse outcomes.

Vitamin B12 supplementation of pregnant Indian mothers led to an improvement in fetal growth. Preconceptional supplementation trials are ongoing and will investigate effect on fetal growth, body composition and long term risk of diabetes and cardiovascular disease. OMICs studies will evaluate the epigenetic effects of interventions.

O12 Jean-Louis Gueant
Markers of folate and vitamin B12 status are associated with insulin resistance and metabolic syndrome severity in morbidly obese patients
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Introduction: Low Vitamin B12 and high folate concentrations during pregnancy are associated with an increased risk of obesity and insulin resistance in offspring. In the general population, high folate is associated with exacerbation of the metabolic markers of vitamin B12 deficiency. In contrast, the influence of vitamin B12 and folate and related markers of the one carbon metabolism on insulin resistance and metabolic syndrome components remains unclear in severely obese patients.

Aim: To evaluate the association of folate, vitamin B12, homocysteine and methyl malonic acid with insulin resistance (HOMA-IR) and the other components of metabolic syndrome in severely obese patients.

Methods: 278 consecutive obese patients were assessed prospectively during the preoperative multidisciplinary evaluation (period 1) and before bariatric surgery (period 2).

Results: We reported the highest HOMA-IR in patients with highest tertile of RBC and either lowest tertile of plasma B12 or highest tertile of methyl malonic acid (p<0.034 and 0.011, respectively). Lg
HOMA-IR was negatively correlated with Lg homocysteine (p>0.0001) and positively correlated with Lg serum folate (p>0.001). In multivariate analysis, HDL-cholesterol and RBC folate remained independent predictors for HOMA-IR at period 1 (p=0.034 and p=0.008, respectively). The independent predictors for HOMA-IR values at period 2 were either BMI and homocysteine (model 1 without serum folate, p=0.010 and p=0.002, respectively) or BMI and methyl malonic acid (model 2 without homocysteine, p=0.030 and p=0.004, respectively). Age and RBC folate remained independently associated with the number of metabolic syndrome components (p= 0.006 and 0.020, respectively).

**Discussion:** RBC folate, homocysteine, and methyl malonic acid are significant predictors of insulin resistance and number of metabolic syndrome components in severely obese patients. An influence of markers of B12 deficit is observed in patients with high folate status. These findings may have important implications for the management of severely obese patients.

**Conflicts of interest**: The authors have no conflict of interest to declare.

**O13 Viktor Kožich Profiling of folates and related sulfur amino acids in SHR rat model of metabolic syndrome**

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**Background:** We reported previously that the spontaneously hypertensive rats—SHR, a model of metabolic syndrome—carry a deletion in the folate receptor 1 (Folr1) promoter which leads to decreased renal expression of Folr1 mRNA, impaired renal folate reabsorption and low serum folates, and presence of several features of the metabolic syndrome. In this study we analyzed the profiles of folates and selected sulfur compounds in SHR and SHR.BN-chr.1 congenic animals (carrying the wild type Folr1 gene) on a regular diet.

**Methods:** Distribution of differentially substituted folate species and the extent of glutamylation in plasma and liver in SHR (n=6-10) and SHR.BN-chr.1 (n=6-9) animals were determined by LC-MS/MS. Concentrations of total aminothiols and thioethers were measured by HPLC and LC-MS/MS, respectively.

**Results:** Plasma folates were significantly lower in the SHR strain compared to SHR.BN-chr.1 (median 44 nmol/L vs. 56 nmol/L, p<0.009), 5-methyltetrahydrofolate (5mTHF) monoglutamate was the predominant form in both strains (>99% of total folate). The SHR animals exhibited significantly higher concentrations of plasma total cysteine and homocysteine, γ-glutamylcysteine and lanthionine than the SHR.BN-chr.1 counterparts. In the liver, the SHR strain- compared to the congenic rats- exhibited a significantly decreased proportion of 5mTHF, an increased proportion of dihydrofolate, tetrahydrofolate and formyltetrahydrofolate, and complex changes in the extent of glutamylation of folate forms. It is unclear whether the imbalance in folate forms in SHR may have any impact on purine and thymidylate biosynthesis, and whether it may lead to any epigenetic consequences.

**Conclusion:** This ongoing study shows that the whole body homeostasis of folates and related sulfur amino acids is substantially impaired in the SHR model and suggests the role of these interconnected pathways in the development of metabolic syndrome.

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**O14 Caitlin G. Howe Genetic variation in one-carbon metabolism, prenatal air pollution exposure, and cardiovascular phenotypes in children**

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**Introduction:** We have previously observed that genetic variation in epigenetic reprogramming genes modifies the effects of prenatal ozone exposure on adverse cardiovascular phenotypes in children. Polymorphisms in one-carbon metabolism (OCM) genes have also been shown to modify the pro-atherogenic effects of particulate matter (PM) air pollution, but this has only been evaluated in adults.

**Aim:** To investigate whether OCM gene polymorphisms modify the effects of prenatal PM exposure on cardiovascular phenotypes in 11 year-old participants (n = 493) from the Southern California Children’s Health Study.

**Methods:** We selected 13 OCM polymorphisms, which 1) have been associated with adverse cardiovascular phenotypes in adults or 2) alter dietary requirements for choline, which is an important source of the pro-atherogenic metabolite trimethylamine N-oxide and is also critical for hepatic secretion of very low-density lipoprotein. Using cross-product terms in mixed effects linear regression models we examined interactions between prenatal PM exposure and OCM polymorphisms in relation to three cardiovascular phenotypes: carotid intima-media thickness, blood pressure, and C-Beta, an indicator of carotid arterial stiffness. Models were adjusted for genetic ancestry, age, BMI, and sex (included as fixed effects) and also community (included as a random effect).

**Results:** There were no overall associations between prenatal PM exposure and cardiovascular phenotypes. However, there was a negative interaction between prenatal PM exposure and a single nucleotide polymorphism (SNP) in *PEMT* (rs12325817), which was statistically significant after adjusting for multiple tests (P_{Bonferroni} = 0.0066). Among children without the rs12325817 variant only (n = 160), a 2 standard deviation increase in prenatal PM exposure (18.89 μg/m³) was associated with an 11% increase in C-Beta (P = 0.0238).

**Discussion:** Our findings suggest that rs12325817, a SNP that has been shown to reduce *PEMT* expression and to increase dietary choline requirements, may protect against PM-induced arterial stiffness in children.

**Conflicts of Interest:** The authors declare that they have no conflicts of interest.

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**O15  Mads Vendelbo  The effect of folic acid supplementation on insulin sensitivity and type 2 diabetes – A meta-analysis of randomized controlled trials**

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**Background:** Supplementing with folate lowers circulating homocysteine and affects other metabolites in the one-carbon metabolic pathway. Various mechanisms link lower homocysteine and one-carbon metabolism to insulin resistance and development of type 2 diabetes (T2D). However, results from randomized controlled trials (RCT) examining the link between folic acid supplementation and measures of T2D risk are inconsistent, potentially because of differences in study population, dose, and duration of the RCTs.

**Methods:** We conducted a systematic literature search in PubMed and Web of Science to identify RCTs assessing fasting glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), glycated hemoglobin (HbA1c) or risk of T2D. The meta-analysis was conducted using both fixed and random-effects models to calculate weighted mean differences or risk ratio with 95% CI.

Results: In total, 25 pertinent studies were identified of which 22 individual studies reported values for fasting glucose, 11 fasting insulin and HOMA-IR and 6 studies reported HbA1c. Additionally two studies reported on T2D development. We found no effect of folate supplementation compared to placebo on fasting glucose (mean difference: -0.02 mmol/L, CI95% [-0.06; 0.02], p=0.41) or HbA1c (- 0.12 %, [-0.35; 0.12], p=0.34). Fasting insulin and HOMA-IR decreased with folate supplementation compared to placebo (- 9.19 pmol/L, [-13.96; -4.41], p<0.001 and -0.57 units, [-0.76; -0.37], p<0.0001 respectively). In T2D patients, we found that folate supplementation lowered fasting plasma glucose (-0.53 mmol/L, CI95% [-1.00; -0.05], p=0.03) compared to placebo. No change in overall risk of developing T2D was seen with
Conclusion: Our results suggest a link between folate supplementation and improved glucose metabolism, although the size of the effects was marginal. Few studies have examined folate supplementation on T2D risk and glucose metabolism related outcomes, and future studies on folate supplementation should include analysis of glucose metabolism parameters and T2D risk to verify this potential effect.

SESSION 6: Animal models for B vitamin-related pathologies

O16 Edward V. Quadros
Role of folate receptor antibodies in neuro-developmental disorders

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The pathogenesis of autism spectrum disorders (ASD) is not known. Multiple causes have been implicated in the etiology of this heritable disorder but no link has been established with a specific gene defect. Maternal autoantibodies against folate receptor alpha (FRα) have been associated with neural tube defect pregnancy, subfertility, preterm birth and fetal abnormalities. Their presence in one or both parents and in the offspring has been associated with developmental disorders including autism in the child. These autoantibodies target FRα and block folate transport to the fetus during pregnancy and in infants block folate transport to the brain. Treating children with oral high-dose folinic acid has shown significant improvements in the core deficits related to autism spectrum disorders, such as language/speech and social interaction.

Preliminary studies in a rat model of exposure to FRα antibody during gestation and the pre-weaning period have confirmed the deleterious effects of the antibodies. Administering antibody to pregnant dams on GD 8 blocks folate transfer from mother to the fetus and decreases folate uptake in the fetus. Antibody accumulates in placenta and induces localized inflammation. Antibody exposed pups when tested for ultrasonic vocalization on PND4 and behavior between PND 40 and 70 showed significantly fewer vocalizations and severe learning and cognitive impairments respectively in otherwise normal appearing rats. This behavioral phenotype is transferred to subsequent generations. Protection from antibody induced behavioral and cognitive deficits is provided by folinic acid and dexamethasone treatment at the time of antibody exposure. Folinic acid can be transported via the alternate reduced folate carrier and dexamethasone can prevent damage to FRα by reducing antibody-mediated inflammation, thus allowing for increased transplacental transport of folate. This has implications in the treatment and prevention of developmental disorders associated with FRα autoantibodies.

Nearly 40% of parents of autistic children are positive for the FRα autoantibodies and over 65% of children with autism are positive for the FRα antibodies. In these children the antibody could block folate transport to the brain. Folinic acid has shown great potential to improve many of the core symptoms of ASD. These initial observations are now confirmed in a double-blind placebo controlled study. While risk / benefits of high dose folinic acid need consideration, treating men, women and infants positive for the antibody, with folinic acid may be beneficial in reducing the incidence of autism and other developmental and pregnancy related abnormalities.

Clinical studies collaborators: Vincent Th Ramaekers, University of Liege Autism Center, Belgium; Richard E Frye, Autism Center, Children’s Hospital, Arkansas, USA. Laboratory contributors: Jeffrey M Sequeira; Maria-Isable Berrocal-Zaragoza; Ankuri Desai.

O17 Frank Rutsch
Successful generation of a neuronal conditional Lmbrd1 knockout mouse

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Introduction: The rare inborn cblF defect of cobalamin (vitamin B₁₂) metabolism is characterized by decreased function of the cobalamin dependent enzymes methionine synthase and methylmalonyl-CoA mutase leading to combined methylmalonic aciduria and hyperhomocysteinemia due to
defective cobalamin efflux from lysosomes. We identified mutations in the *LMBRD1* gene as the cause of the cblF defect, which encodes for the lysosomal membrane protein LMBD1. We have previously shown by cross breeding *Lmbrd1<sup>flox3/neo</sup>* with Cre-deleter (PGK-Cre) mice that complete knockout of Lmbrd1 is embryonic lethal because of defects in gastrulation (1). Defects of lysosomal transport mechanisms are associated with neurodegenerative processes and therefore with common diseases such as M. Alzheimer.

**Aim:** We hypothesize that LMBD1 plays an important role in neuronal development and that neuronal loss-of-function of LMBD1 results in neurodegenerative processes.

**Methods:** To generate a conditional *Lmbrd1*-knockout mouse we first characterized the specific expression pattern of *Lmbrd1/LMBD1* in mouse neuronal tissues by *in situ* hybridization and immunohistochemistry.

**Results:** We demonstrated *Lmbrd1* expression in neuronal tissues during embryonic development and postnatally in the brain in wild type mice. We confirmed our expression studies by immunohistochemical staining of LMBD1. By cross breeding *Lmbrd1<sup>flox3/neo</sup>* mice with CamKII mice, we then generated a neuronal conditional *Lmbrd1* knockout mouse.

**Conclusion:** With this animal model, we expect to gain new insights into the function of lysosomal cobalamin transport during neuronal development and in neurodegenerative processes.

**Conflicts of interest:** None declared.


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O18  
Tomas Majtan  
Engineering and characterization of an enzyme replacement therapy for classical homocystinuria

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**Introduction:** Classical homocystinuria (HCU) is an inborn error of sulfur amino acid metabolism caused by the loss of cystathionine beta-synthase (CBS) activity and consequent accumulation of homocysteine and depletion of cysteine. Current treatments are suboptimal and hard for patients to follow. Thus we have initiated the development of an enzyme replacement therapy based on PEGylated human truncated CBS (PEG-CBS).

**Aim:** To rank-order several PEG-CBS conjugates for their efficacy to correct plasma metabolite profile of murine homocystinuria after multiple rounds of repeated administration interrupted with washout periods and to characterize the most promising PEG-CBS candidate(s).

**Methods:** The stable-isotope-dilution gas chromatography mass spectrometry was used for determination of sulfur amino acids in plasma samples from HO mouse model injected with various PEG-CBS conjugates. Various biochemical and proteomic techniques were employed to characterize the most promising PEG-CBS candidate(s).

**Results:** Attenuation of potency, most likely due to immune response, was observed warranting an engineering and characterization of various PEG-CBS conjugates. We found that CBS coupling with maleimide PEG, targeting the residues C272 and possibly C275, inconsistently and insufficiently modified the enzyme yielding two major forms and traces of an unmodified enzyme. On the other hand, the PEG- CBS conjugate with 20 kDa linear NHS ester-activated PEG showed very little loss of potency in vivo most likely due to a complete and reproducible PEGylation resulting in high molecular weight species modified with five PEG chains per subunit on average. We also developed the assays suitable for monitoring and evaluating extent of the CBS PEGylation and demonstrated a sustainable partial normalization of murine homocystinuria upon continuous administration of PEG-CBS via osmotic pumps.

**Discussion:** Administration of 20 kDa NHS PEG-CBS to homocystinuric HO mice results in sustainable and substantial correction of plasma metabolites. Thus we identified the PEG-CBS conjugate suitable for manufacturing and clinical development.

**Conflict of interest:** The research was funded by Orphan Technologies Ltd., a private pharmaceutical company developing an enzyme replacement therapy for CBS-deficient homocystinuria. TM and JPK are inventors on patents related to the processes and products referred here (US patents 9,034,318
O19 Hieronim Jakubowski  
Transcriptional Repression of Male-specific MUP20 Expression in Female Mice is relieved by the Inactivation of Cystathionine-β Synthase Gene

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Introduction: In humans, hyperhomocysteinemia (HHcy) is associated with pregnancy complications including early pregnancy loss, preeclampsia, and congenital birth defects. In mice, HHcy due to cystathionine-β synthase (Cbs) deficiency causes female, but not male, infertility. A family of small, related proteins, Major Urinary Proteins (MUPs), is important for sexual signaling in mice. MUPs are expressed in the liver and excreted in urine at ~10 mg/mL. Male and female mice differ in MUP patterns and concentrations in their urine. A male-specific MUP20 that attracts females is absent in female mice.

Aim: Our aim was to test a hypothesis that female infertility in Cbs⁻/⁻ mice is caused by deregulated expression of MUPs and to examine an underlying mechanism.

Methods: ESI-mass spectrometry, SDS-PAGE, and Western blotting with polyclonal anti-MUP1 antibodies was used to identify and quantify MUP in urine and liver of Tg-I278T-Cbs⁻/⁻ mice (n=10) and Cbs⁺/- sibling controls (n=10). We used RT-qPCR to quantify MUP mRNA.

Results: Total urinary MUP levels were reduced ~2-fold in Cbs⁻/⁻ mice vs. Cbs⁺/- controls, both females (12.0±0.9 vs. 5.9±2.0 mg protein/mM creatinine, p=0.0014) and males (4.9±1.5 vs. 2.3±1.1 mg protein /mM creatinine, p=0.056). A male-specific MUP20 was expressed in Cbs⁻/⁻, but not in Cbs⁺/-, female urine (Figure). In contrast, in male urine, MUP20 level was not affected by Cbs genotype. Levels of other MUPs were reduced in Cbs⁻/⁻ male and female urines (Figure). In livers, MUP20 protein and mRNA were expressed in Cbs⁻/⁻, but not Cbs⁺/-, females. Protein and mRNA levels of other MUPs were reduced in Cbs⁻/⁻ mouse liver, both male and female.

Discussion: Our findings show that a transcriptional mechanism represses the expression of male-specific MUP20 in female mice. Deregulation of this mechanism in HHcy causes masculinization of female Cbs⁻/⁻ mice and may contribute to their infertility.

Conflicts of interest: The authors declare no conflict of interest. Supported by NCN grants 2012/07/B/NZ7/01178, 2013/09/B/NZ5/02794, NCN 2014/15/N/NZ5/01646.

O20 Nicholas D.E. Greene  
One-Carbon Metabolism and Neural Tube Closure Defects

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Introduction: Folate one-carbon metabolism (FOCM) is implicated in the causation of neural tube defects (NTDs), severe birth defects of the developing central nervous system, including spina bifida and anencephaly. Sub-optimal maternal folate status is a risk factor, folic acid supplementation
prevents some NTDs and genetically-determined abnormalities of FOCM within the embryo may contribute to NTD predisposition. Questions remain however concerning the mechanisms linking maternal folate status and NTD risk in the offspring and the functions of FOCM that are required within the embryo for neural tube closure. Mthfr mediates partitioning of one-carbon units between the methylation and folate cycles.

**Aim:** The purpose of this project is to investigate the key functions of FOCM in neural tube closure and to understand the mechanisms by which perturbation of FOCM causes NTDs.

**Methods:** We examined the effect of genetic, pharmacological and nutritional interventions on neural tube closure in mouse embryos and analysed the profiles of multiple folates and methylation cycle intermediates by mass spectrometry (LC-MS/MS).

**Results:** Genetic ablation of **Gldc** (encoding glycine decarboxylase) causes abnormal folate profile and NTDs, that are preventable by supplemental formate. This is consistent with NTDs being caused by insufficient supply of 1C units from mitochondrial FOCM. Inhibition of S-adenosylmethionine production causes NTDs in cultured mouse embryos and we tested whether this was due to impaired methylation or imposition of a ‘methyl trap’. In contrast, knockout of **Mthfr** null embryos does not cause NTDs, despite lack of 5-methyl THF production. **Mthfr** and **Gldc** mutations exhibit genetic interaction in which the frequency of **Gldc**-associated NTDs are altered by **Mthfr** genotype.

**Discussion:** Neural tube closure depends on supply of 1C units from mitochondrial FOCM. Integrity of the methylation cycle is also essential for neural tube closure, but does not depend on supply of 1C units from embryonic folate metabolism.

**SESSION 7: Nutrition during pregnancy and infancy – pathology and physiology**

**O21** Anne-Lise Bjørke- Monsen their infants.

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**Introduction:** Pregnancy and lactation are reported to alter maternal cobalamin status.

**Aim:** Study normal variations in maternal plasma B vitamin status during pregnancy and lactation.

**Methods:** We have measured plasma B vitamins, i.e. vitamin B1 (thiamin and TMP), B2 (riboflavin and FMN), B3 (nicotinamide and mNAM), B6 (PLP), B9 (folate), B12 (cobalamin) and the metabolic markers tHcy, MMA and HK/XA, in healthy, non-pregnant (n=158), pregnant and lactating women and their infants at 6 months (n=114).

**Results:** Compared to non-pregnant women, maternal TMP, FMN, PLP, folate and cobalamin levels decrease, while maternal thiamin, riboflavin and mNAM levels increase from pregnancy week 18 to 36. TMP, mNAM and folate were normalized at 6 weeks postpartum, while thiamin, riboflavin, PLP and cobalamin levels were substantially higher postpartum compared to non-pregnant women. In line with the decrease of the associated vitamins, tHcy, MMA and HK/XA increased from pregnancy week 18 to 36 and normalized during the postpartum period. Infant riboflavin, FMN, PLP, cobalamin, tHcy, MMA and HK/XA levels at age 6 months were essentially equal to levels in non-pregnant women, whereas all other B vitamins and metabolic markers were substantially higher.

**Discussion:** Pregnancy and lactation are associated with an increased risk of maternal B vitamin deficiency. Profound physiologic changes hampers evaluation of maternal vitamin status and limit the use of reference ranges based on non-pregnant women. Effort should be made to establish adequate cut off levels for maternal B vitamins during pregnancy and lactation in order to secure an adequate maternal status during these important periods.

**Conflict of interests:** The authors have no financial relationships relevant to this article to disclose. Dr. Ueland and Dr. Bjørke-Monsen are members of the Steering board of the nonprofit Foundation to Promote Research into Functional Vitamin B12 Deficiency. Dr. Varsi, Arve Ulvik and Øivind Midttun have no conflicts of interest relevant to this article to disclose.
Parental one carbon metabolism, pregnancy outcome and child development and health

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Routine preventive measures to achieve an optimal outcome at birth are limited by the short window of time marked by the duration of pregnancy. The physio pathological process leading to impaired placental implantation and function occurs during early pregnancy. However, most pregnancy studies are performed after the maternal-placental-foetal unit has been fully established, during, the second half of pregnancy. Maternal status in folate, cobalamin and other nutrients involved in one carbon metabolism are determining factors in the prevention of adverse pregnancy outcome and with lasting effects on child development. The role of folic acid in preventing neural tube defects is well established. Moderately elevated maternal tHcy at preconception is negatively associated with neurological development in the offspring at 4 months and 6 years. However, there is scarce data regarding the effect of paternal one carbon metabolism, biological and lifestyle factors on the placentation process, functioning of the maternal-placental-foetal unit and on pregnancy outcome.

The Reus Tarragona Birth Cohort is an ongoing cohort study investigating the role of maternal and paternal nutrient, genetic and lifestyle components on placental vascular function, pregnancy outcome and on health and development in the child. Mothers with a confirmed pregnancy and a viable foetus, followed by first prenatal blood draw before 12 gestational weeks are eligible to participate. Regarding interactions between nutrients involved in one carbon metabolism, results from 562 mother-new born dyads indicate lasting effects of first trimester folate-cobalamin interactions on one carbon metabolism and haematological parameters throughout pregnancy. The role of dimethylglycine in homocysteine regulation is enhanced when folate status falls during pregnancy. Preliminary results based on 308 mother-father-new born triads indicate that both the maternal and paternal one carbon metabolic network influence placental vascular function. The paternal MTHFR 677TT genotype increases the probability of elevated arterial uterine artery resistance index at 20 GW and of pregnancy induced hypertension, compared to the MTHFR 677CC genotype. Moderately elevated tHcy in either the mother or the father is associated with increased probability of intrauterine growth retardation. Preliminary results from 182 mother-child dyads (aged 7.5 years) reveal associations between different nutritional, genetic and metabolic, components of maternal one carbon metabolism and adiposity and growth in the child at 7.5 years of age and will be discussed.

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Utility of newborn screening markers to diagnose newborns at risk of vitamin B-12 deficiency second to maternal vitamin B-12 deficiency

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Introduction: Maternal vitamin B-12 (B-12) deficiency can lead to offspring B-12 deficiency, which presents with non-specific symptoms and may worsen to long-term health consequences if untreated. Newborn screening (NBS) for B-12 deficiency has been recommended. Current NBS markers, however, may lack sensitivity.
Aim: To explore the utility of NBS markers, including acylcarnitines and amino acids, to identify newborns at risk of B-12 deficiency second to maternal B-12 deficiency.

Methods: This study included 685 apparently healthy mother-newborn dyads from British Columbia, Canada. Maternal serum total B-12, methylmalonic acid (MMA), and total homocysteine (tHcy) concentrations were assessed during the 1st and 2nd trimester [mean (range) 11.5 (8.3-13.9) and 16.5 (14.9-20.9) gestational weeks, respectively]. Acylcarnitines and amino acids were routinely quantified in neonatal dried blood spots (DBSs). Neonatal DBS MMA was additionally analyzed. The performance of NBS markers to diagnose newborns of mothers categorized as B-12 deficient (defined as: total B-12 <148pmol/L with MMA >260pmol/L concurrently at both trimesters; n=41) was evaluated by ROC analysis.

Results: Maternal serum total B-12 and MMA, but not tHcy, concentrations at both trimesters weakly predicted multiple NBS markers related to B-12 metabolism, including propionate-metabolites (C3), MMA, methionine, and valine/phenylalanine (multiple linear regression; P<0.05). The best fitted models (R²: 0.03-0.1; P<0.01) were observed for neonatal C3, C3/C2, methionine, and DBS MMA. Neonatal DBS MMA was the only NBS marker with a significant capacity [AUC (SE): 0.68 (0.06)] to diagnose newborns of B-12 deficient mothers; 24% of newborns of B-12 deficient mothers had DBS MMA above the reference interval (97.5th percentile) of healthy, term newborns.

Discussion: The sensitivity of NBS markers to detect newborns of B-12 deficient mothers appears low. Only small disturbances in NBS markers related to B-12 metabolism are described by maternal B-12 status, which may be explained by a preferential unidirectional transport of B-12 to the fetus.

Conflict of Interest: none to declare.

O24 Carles Lerin Breast milk levels of one-carbon metabolites and infant postnatal growth

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O25 Jacob Selhub Higher Cord Blood Unmetabolized Folic Acid (PGA) Levels Are Associated with the Development of Food Allergy in Children

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Introduction: Food Allergy (FA) prevalence has increased in recent years, but the reasons for this increase are largely unknown. One hypothesis involves changes in our nutrition, including increased exposure to folic acid through fortification and supplement use.

Aim: We examined whether prenatal total folate, 5-methyltetrahydrofolate (5-MTHF) and/or PGA concentrations are associated with the development of food sensitization (FS) and FA in children.

Methods: A nested case control study was performed in the Boston Birth Cohort. Total folate was measured in maternal plasma at delivery by a chemiluminescent immunoassay (New Industrial). 5-MTHF and PGA were measured in cord blood by LC-MS/MS. Children were followed at 6-9 months, 1-2 years and 3-4 years. Food-specific IgE (sIgE) was measured to common food allergens (egg, milk, peanut, soy, wheat, walnut, shrimp, and sesame) using ImmunoCAP. Based on sIgE and clinical history, children were classified as a) FS (sIgE ≥ 0.35kU/L) or not and b) FA or not. Folate levels were divided into quartiles, and multiple logistic regression was used to estimate odds ratios and 95%
Results: A total of 502 children were included in this study: 193 (38%) were FS and 33 (6.6%) were FA. Mean total folate, 5-MTHF, and PGA levels did not differ between those with and without FS, but mean cord blood PGA levels were significantly higher in those who later developed FA (1.73 vs 1.30 nmol/L, p=0.001), with increasing quartiles of PGA associated more strongly with FA (test for trend p=0.001).

Discussion: Among children in the BBC, higher levels of cord blood PGA were associated with the development of FA. Whether this is due to increased exposure to synthetic folic acid in utero or underlying genetic differences should be further studied.

Conflicts of Interest: None

Figure: O26

Martha S. Field

The effect of folate on vitamin B12 depletion-induced inhibition of nuclear thymidylate biosynthesis and neural tube defects

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Introduction: Clinical vitamin B12 deficiency can lead to megaloblastic anemia, which results from inhibition of DNA synthesis by trapping folate cofactors in the form of 5-methyltetrahydrofolate (5-methylTHF) and subsequent inhibition of de novo thymidylate (dTMP) biosynthesis. Observational studies have also shown an association between maternal vitamin B12 deficiency and neural tube defects (NTDs) in both folate-fortified and non-fortified populations. In the cytosol, vitamin B12 functions in the remethylation of homocysteine to methionine, whereas in the nucleus THF is required for de novo dTMP biosynthesis.

Aim: The impact of vitamin B12 depletion on nuclear de novo dTMP biosynthesis and neural tube defect occurrence was investigated in methionine synthase (MTR)-null human fibroblast and nitrous oxide-treated HeLa cell models and in the Shmt1 mouse model.

Results: In vitamin B12 deficient cell culture models, the nucleus was more sensitive to 5-methylTHF accumulation than the cytoplasm, with nuclear 5-methylTHF levels increasing greater than 4-fold. Vitamin B12 depletion decreased de novo dTMP biosynthesis capacity by 5-35%, whereas de novo purine synthesis, which occurs in the cytosol, was not affected. Phosphorylated histone H2AX (H2AX), a marker of DNA double-strand breaks (DSBs), was increased in vitamin B12 depletion, and this effect was exacerbated by folate depletion. Furthermore, vitamin B12 deficiency is an independent risk factor for NTDs in the Shmt1 mouse model, which exhibits impaired de novo dTMP synthesis and develops folic acid-responsive, sporadic NTDs in states of vitamin B12 sufficiency.

Discussion: These findings indicate that a nuclear 5-methylTHF trap occurs in vitamin B12 depletion, which suppresses de novo dTMP biosynthesis and causes DNA damage, accounting for the pathophysiology of megaloblastic anemia observed in vitamin B12 and folate deficiency. Although maternal vitamin B12 deficiency caused NTDs in the Shmt1 mouse model, it did not significantly impair growth or increase plasma homocysteine, indicating that NTD occurrence is more sensitive to vitamin B12 deficiency than homocysteine remethylation.

Conflicts of interest: none
O27  Jean-Louis Guéant  Cellular stress is a key actor of a common scenario, which participates to the high variability of neurological and visceral manifestations of cobalamin disorders

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The deficiency in vitamin B12 (cobalamin, Cbl) and most inborn errors of cobalamin absorption, blood transport and cellular metabolism produce a decreased activity of methionine synthase. This enzyme synthesizes methionine by remethylation of homocysteine. Methionine is the precursor of S-adenosylmethionine (SAM), the methyl donor needed for epigenomic mechanisms.

The high variability of neurological and visceral manifestations of nutritional and inherited Cbl disorders is far from being understood. Recent results from our consortium and other groups suggest that it results in part from a common scenario, in patient fibroblasts, deficient rats, Cd320 KO mouse and cKO mice with Mtr conditional inactivation in brain, liver and heart. Nutritional and inherited Cbl disorders produce oxidant stress through increased production of reactive oxygen species, decreased concentration of glutathione and decreased expression of protective enzymes.

As a consequence, free radicals inactivate methionine synthase activity through cob(I)alamin oxidation and trigger endoplasmic reticulum (ER) stress. Cbl deficiency leads to non-adapted ER stress and mislocalization of stress proteins involved in mRNA trafficking. This produces an altered nucleo-cytoplasmic shuttling of mRNAs, including those from deacetylase SIRT1 and methyl transferases (DNMTs and PRMT). The resulting reduced activity of SIRT1 impairs the expression of heat shock proteins through increased acetylation of heat shock factor 1 (HSF1). The decrease of SAM, SIRT1, DNMT and PRMT produce a decreased expression of genes involved in cell proliferation and differentiation, neuroplasticity, energy metabolism in heart and liver and bone tissue homeostasis through DNA methylation changes and imbalanced methylation/acetylation of PGC-1α, the co-activator of nuclear receptors PPAR-α, ER-α, ERR-α, HNF-4α and VDR.

Taken together these data show that oxidant stress, ER stress, altered nucleo-cytoplasmic shuttling of mRNAs and epigenomics dysregulations are key interacting pathomechanisms of Cbl deficiency and inborn errors of Cbl metabolism and that the decreased SIRT1 activity plays a central role in this scenario.

O28  Luciana Hannibal  Molecular characterization of the CblC disease reveals new pathways in pathogenesis

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Background: Nutritional and functional deficiencies of cobalamin lead to severe neurological and hematological dysfunction, and in severe cases, to death. Intracellular processing of dietary cobalamins is catalysed by the CblC protein, which primes the cobalamin molecule prior to its delivery to cytosolic methionine synthase and mitochondrial methylmalonyl-CoA mutase. Dysfunction or absence of CblC leads to combined hyperhomocysteinemia and methylmalonic acidemia (MMACHC, OMIM 609831) the most common genetic disorder of cobalamin metabolism. In spite of over 500 cases identified to date, little is known about the molecular derangements that characterize cblC disease onset and progression.

Methods: The production of homocysteine (Hcy) and methylmalonic acid (MMA) was examined in cultured fibroblasts from healthy neonates and in 5 genetically unrelated patients with early onset CblC disease. Protein expression levels and the presence of post-translational modifications were identified by SILAC (Stable Isotope Labeling by Amino acids in Cell Culture) and mass spectrometry.

Results: Cells from cblC patients with neonatal onset demonstrated changes in protein expression
levels affecting various facets of metabolism including mitochondrial function and one-carbon metabolism. Our study identified for the first time a number of methylated proteins in Lys, Arg and Glu amino acid residues.

**Discussion:** Data from metabolite, protein expression levels and post-translational modifications concur with the clinical manifestations of the CblC disease and help to explain the insufficient response to treatment with hydroxocobalamin. Our study identifies new pathways in pathogenesis and suggests abnormal proteostasis in the CblC disorder.

**Conflicts of Interest:** The authors declare no conflict of interest.

**O29 David Coelho**  
Methionine synthase and methionine synthase reductase interact with MMACHC and with MMADHC  
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**Introduction:** An increasing number of studies indicate that each step of the intracellular processing of vitamin B12 or cobalamin (Cbl) involve protein-protein interactions. We have previously described a novel interaction between methionine synthase (MS) and MMACHC and its effect on the regulation of MMACHC activity.

**Aim:** Our goal is to further characterize the interactions of MS with other potential partners in a so-called MS interactome.

**Methods:** We dissected the interactions and their alterations by co-immunoprecipitation and DuoLink proximity ligation assays in fibroblasts with cblG, cblE, and cblC genetic defects affecting respectively the expression of MS, methionine synthase reductase (MSR) and MMACHC and in HepG2 cells transfected with corresponding siRNAs.

**Results:** We observed the known interactions of MS with MSR and with MMACHC as well as MMADHC with MMACHC, but we also observed novel interactions for MSR with MMACHC and with MMADHC and in HepG2 cells transfected with siRNAs. Our data show that the processing of Cbl in cytoplasm occurs in a multiprotein complex composed of at least MS, MSR, MMACHC and MMADHC, which could contribute to shuttle safely and efficiently Cbl towards MS.

**Discussion:** Our data suggest that defective protein-protein interactions among key players of this pathway could contribute to the molecular mechanisms of the cblC, cblG and cblE genetic defects and provide novel insights into our understanding of the pathophysiology of inherited disorders of Cbl metabolism.

**Conflicts of Interest:** The authors declare no conflict of interest.

**O30 Patrick J. Stover**  
Arsenic Targets Folate-dependent de novo Thymidylate Synthesis in the Nucleus Leading to Neural Tube Defects  
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**Introduction:** Arsenic exposure increases risk for cancers and is teratogenic in animal models.

**Aim:** Here we demonstrate that Small Ubiquitin-like Modifier (SUMO)- and folate-dependent nuclear de novo thymidylate (dTMP) biosynthesis is a sensitive target of arsenic trioxide (As2O3), leading to uracil misincorporation into DNA and genome instability.

**Results:** Methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) and serine hydroxymethyltransferase (SHMT) generate 5,10-methylenetetrahydrofolate for de novo dTMP biosynthesis and translocate to the nucleus during S-phase where they form a multi-enzyme complex with thymidylate synthase (TYMS) and dihydrofolate reductase (DHFR) as well as the
components of the DNA replication machinery. As2O3 exposure increased MTHFD1 SUMOylation in cultured cells and in in vitro SUMOylation reactions, and increased MTHFD1 ubiquitination and MTHFD1 and SHMT1 degradation. As2O3 inhibited de novo dTMP biosynthesis in dose-dependent manner, increased uracil levels in nuclear DNA, increased genome instability, and caused neural tube defects in embryos.

Discussion: These results demonstrate that MTHFD1 and SHMT1, which are key enzymes providing one-carbon units for dTMP biosynthesis in the form of 5,10- methylenetetrahydrofolate, are direct targets of As2O3-induced proteolytic degradation, providing a mechanism for arsenic in the etiology of cancer and developmental anomalies.

COI: None

O31 Sergey A. Krupenko
Proteasomal degradation of ALDH1L1 during the transition from G0/G1 to S phase

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Introduction. ALDH1L1 (10-formyltetrahydrofolate dehydrogenase) is an abundant folate enzyme, which is involved in the regulation of cellular proliferation. It is ubiquitously silenced in cancer cell lines through the promoter methylation but is expressed in NIH3T3 cells. We observed, however, that levels of ALDH1L1 in NIH3T3 cells are fluctuating through the cell cycle: they are increased in confluent cell culture but become undetectable in proliferating cells.

Aim. We have studied the mechanism of Aldh1l1 degradation in NIH3T3 cells.

Methods. Cell cycle analysis, in vivo and in vitro ubiquitination assays, and protein pull-down were employed. Assays of reduced folate pools and NMR-based targeted metabolomics were used to characterize cell cycle-dependent metabolic changes.

Results. Evaluation of ALDH1L1 in cells synchronized at different phases of the cell cycle demonstrated that its expression is associated with the quiescent state (G0/G1) while cells in the S phase lack the protein. A pull-down technique in combination with LC/MS-MS analysis has identified 17 targets from the ubiquitin-proteasome pathway as ALDH1L1-interacting proteins, suggesting that the rapid clearance of ALDH1L1 during the transition from G1 to the S phase is associated with the proteasome-dependent degradation. In support of this hypothesis, we have demonstrated that the treatment of cells with the proteasome inhibitor MG-132 prevented the drop of ALDH1L1 levels while confocal microscopy revealed co-localization of ALDH1L1 with proteasomes. We have further identified STUB1 protein (E3 ubiquitin-protein ligase CHIP) as the E3 ligase ubiquitilating ALDH1L1. Our data also indicate that HSP90 could be the adaptor protein mediating the ubiquitination of ALDH1L1 by STUB1.

Discussion. Analysis of the levels of reduced folates and NMR-based profiling of major metabolites in different phases of the cell cycle provided clues for such stringent regulation of a major folate enzyme from the metabolic point of view.

The author declares no conflict of interest.

SESSION 9: Nutrition and disease

O32 Alex Brito & Lindsay H. Allen
New perspectives on vitamin B-12 deficiency in low income populations; assessment and prevalence

Alex Brito1 & Lindsay H. Allen2.

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Vitamin B-12 deficiency occurs primarily as a result of insufficient dietary intake of animal source foods which are the only natural source of the vitamin. Deficiency is therefore common in low- and middle-income countries (LMIC) where animal product consumption is lowest. Population assessment has been limited to serum or plasma B-12. Worldwide data indicates that prevalence of low serum or plasma B-12, based on conventional cut points for deficiency (<148 pmol/L) and marginal (148-221 pmol/L) status combined, varies widely, in some countries exceeding 40% in
specific population groups (children, young adults, pregnant women, and elderly). The high prevalence of low serum B-12 is often accompanied by high folate status due to mandatory folic acid fortification of staple foods. Incorporation of available metabolic B-12 status biomarkers (MMA, tHcy and holoTC), validation of cut points, application of the combined indicator of B-12 status (cB-12), and assessment of folate-B12 interaction, are needed to accurately assess the prevalence and consequences of B-12 deficiency and determine the magnitude of deficiency as a public health problem. Our presentation will provide new calculations of the prevalence of B-12 deficiency based on both single B-12 biomarkers and cB-12. The potential value of using breast milk vitamin B12 concentration as a population status biomarker will be proposed based on data from populations with a range of maternal B12 status. Comparisons between wealthier countries and LMIC will be presented and the interaction of high folate and low B12 status considered. Validation of cut points for individual B-12 markers and adjustments of cB-12 based on analyses conducted from a database of 11 prospective cohorts in the perinatal period will be presented. This presentation will encourage more sophisticated estimates of B-12 deficiency and its consequences in poor and middle-income populations.

O33  Sadanand Naik  Cow milk, buffalo milk or vitamin pills for improving vitamin B12 status: A four week prospective study

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Aim: To compare the effect of daily consumption for four weeks of cyano-B12 supplementation with consumption of cow and buffalo milk containing equivalent amounts of endogenous protein bound hydroxo-B12

Methods: Adults (n=53, 32 females) were supplied for four weeks with 2 x 0.8 μg vitamin B12 (B12) daily; either as cyano-B12 in tablets (n=17, 6 females) or as hydroxo-B12 naturally present in 200 ml of cow (n=19, 14 females) or buffalo (n=17, 12 females) milk. We measured plasma B12, holotranscobalamin (holoTC) and homocysteine (Hcy) at baseline and every second week. The differences between the values at baseline minus two-week or four-week (“delta”) levels were compared. Statistical difference from the baseline was assessed for each marker by Wicoxon ranked test for the median (p in red). Difference in response between the groups (e.g. tablets vs cow’s milk) was examined by Wilcoxon test for unpaired data (p in green).

Results: Figure 1 summarizes the results presented as the difference from the baseline at weeks 2 and 4. The three groups show a comparable increase in total B12. A significant increase in holoTC was observed only for the milk treatments (week 2) but this response levelled off at week 4. Changes in Hcy in the two milk groups reversed mirrored the holoTC changes. Response in Hcy in the tablet group appeared after 4 weeks. The results may be (at least in part) affected by some differences in the baseline values.

Conclusion: Results confirm that milk is a highly bioavailable and suitable source of B12, but do not convincingly document any superiority of milk as compared to the vitamin pills. No difference between the two types of milk was observed.
Genetic epidemiology of static and functional biomarkers of vitamin B₁₂ status in older adults

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Introduction: Plasma vitamin B₁₂ (B₁₂) levels among older adults is highly variable and significantlyheritable. Epidemiological studies of static (plasma B₁₂ and holoTC) and functional biomarkers (MMA and homocysteine) of vitamin B₁₂ have shown that increasing age, gender, renal function, and serum/plasma levels of folate and vitamin B₁₂ to be important drivers of population variability in these biochemical parameters in older adults.

Aim: We carried out a twin study to (i) characterise the basis of inter-individual variation in biomarkers commonly used to assess B₁₂ status, namely plasma B₁₂, holoTC, MMA and homocysteine as well as in plasma folate from 368 healthy, older, female twins; and (ii) to test if common genetic variation in B₁₂ candidate genes are associated with variability in a combined indicator of B₁₂ status (cB₁₂).

Methods: We used the computer package Mx to partition the relative contribution of heritable (h²), and environmental (common & unique) factors to the observed total variance of each biomarker and the composite score. We used PLINK to test for association between variability of each biomarker, the composite score and candidate polymorphisms in genes: FUT2, MUT, ABCD4, CUBN, TCN1, HIBCH, and ACSF3.

Results: We show that variability in B₁₂, holoTC, homocysteine levels and cB₁₂ score were highlyheritable (h²= 55%-64%), while variability in folate and, surprisingly, MMA concentrations is due to environmental factors. Candidate polymorphism study showed that SNPs in genes CUBN (P<0.05, r²~2%) and HIBCH (P<2.3E-04, r²~9%) are associated with variation in the cB₁₂ score.

Discussion: Our study suggests that variability in B₁₂ status in healthy, older adults is highly heritable but environmental factors also influence some B₁₂ markers. Genetic loci known to be associated with MMA are also associated with the cB₁₂ score.

Conflicts of interest: None.

Vitamin B₁₂ deficiency affects distinct pathways in males and females

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Introduction: A significant proportion of Indian population, adhere to a strict vegetarian diet and hence have low vitamin B₁₂ levels. Most importantly, more than 70% of pregnant women in India are vitamin B₁₂ deficient. In-utero micronutrient deficiencies could lead to development of metabolic disorders like obesity, cardiovascular diseases in later stages of life which might be due to epigenetic changes. Deficiency of Vitamin B₁₂, a key player in the one-carbon metabolism, could alter DNA methylation and hence protein expression which could lead to the development of complex disorders.

Aim: To evaluate if maternal vitamin B₁₂ deficiency leads to increased atherogenic risk in the offspring (males and females).

Methods: Female wistar rats were fed B₁₂ deficient diet for 3 months and mated with control male rats. To identify differentially expressed proteins in liver of male and female pups, 4-plex iTRAQ experiments were performed in triplicates. To prove causality vitamin B₁₂ was supplemented to a subset of mothers (on restricted diet) at conception or parturition.

Results: On an average of 2313 and 2253 proteins were identified in males and females respectively (n=3) at 1% FDR. Of these, 239 and 317 proteins were differentially expressed in males and females.
respectively. Only 29 proteins were common between males and females. Proteins involved in fatty acid, amino acid and energy metabolism were predominantly altered. Interestingly, PPAR-alpha was found to be downregulated in males but up regulated in females while CSAD, an enzyme in the taurine metabolism pathway was severely down regulated in females.

**Discussion:** Our results indicate that PPAR-alpha is altered due to vitamin B$_{12}$ deficiency which could explain increased triglyceride and low ApoA1 and HDL levels in male but not in female pups. Low CSAD leading to low taurine could lead to defect in bone mineralization thus leading to bone related diseases.

**Conflicts of interest:** None Declared.

![Figure describing distinct pathways altered in males and females under vitamin B$_{12}$ deficiency.](image)

**SESSION 11: Cognition, dementia, and B vitamins – animal models, trials and epidemiology**

**O36**  
A. David Smith  
How does the brain mediate the effects of low vitamin B12 and raised homocysteine on cognition?

A. David Smith.  
Oxford Project to Investigate Memory and Ageing (OPTIMA), Department of Pharmacology, University of Oxford, UK.

Elevated plasma concentrations of tHcy or low concentrations of vitamin B12 are associated with impairment of cognition and may lead to dementia. Is it possible to identify a common mechanism for these associations? I will argue that impairment of methylation processes may be one of the key biochemical initiation steps and will show how this can lead to a variety of damaging effects in the brain. Inadequate supply of methionine and/or inhibition of a variety of methylation reactions leads: to impairment in transmission of signals from one brain region to another, so disrupting neural networks; to impairment in synaptic functions mediating cognition; to the formation of Alzheimer-type neuropathology; to the death of neurons and to regional brain atrophy. Some of these effects may be prevented by B vitamin supplementation, but if they are not prevented then the outcome is likely to be dementia.

**Key references:**  

**O37**  
Aron M. Troen  
Homocysteine and the Brain-Body divide

Aron M. Troen.  
The Nutrition and Brain Health Laboratory, The Institute of Biochemistry Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture Food and Environment, The Hebrew University of Jerusalem, Israel.
Evidence from randomized clinical trials, with respect to the ability of B-vitamin supplements to lower circulating homocysteine and thereby to improve cognitive health in older adults, has been inconsistent. While most trials have been null, a handful show a significant treatment benefit. Opinions differ as to the reasons for the lack of overall efficacy, and the distinguishing features of the positive trials. In retrospect, some inconsistency may be explained by the considerable heterogeneity between trials, and by differences between treatment-responsive and non-responsive individuals within the trials. Another possibility is that unless it is assumed that elevated homocysteine and disrupted metabolism in the periphery reflect brain metabolism, then lowering homocysteine in circulation need not affect the brain. Both possibilities are consistent with the data from animal models of hyperhomocysteinemia, which clearly show that homocysteine metabolism in brain and periphery are not predictably linked. The literature also shows inconsistent and heterogeneous neurological, cerebrovascular and cognitive outcomes in vivo, under different experimental and metabolic conditions. If, as the data suggest, circulating homocysteine is a non-specific biomarker for different metabolic states with different brain consequences, then improving treatment outcomes will hinge upon improving our understanding of the metabolic interactions between brain and body that mediate cognitive decline.

Conflicts of Interest: None

O38 Jeffrey M. Sequeira Behavioral alterations associated with vitamin B12 deficiency in the TCblR/CD320 KO mouse
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Introduction: Vitamin B12 (Cobalamin, Cbl) deficiency is prevalent worldwide and causes megaloblastic anemia and neurologic deficits. While the anemia can be treated, the neurologic deficits can become refractive to treatment as the disease progresses. Therefore, timely intervention is critical for a favorable outcome. Moreover, the metabolic basis for the neuro-pathologic changes and the role of Cbl deficiency in the pathology still remains unexplained.
Aim: Using a CD320 KO mouse that lacks the receptor (TCblR) for uptake of transcobalamin (TC) bound Cbl, we investigated whether Cbl deficiency in the central nervous system (CNS) produced functional neurologic deficits in the mouse that could parallel those observed in humans.
Methods: Behavioral testing included the open field test, marble burying test and place avoidance testing, including assessment of behavioral flexibility. Anatomical analysis included measurements of brain weights and size of nucleus of hippocampal pyramidal neurons of WT and KO mice. Synaptic physiology analyses were focused on the hippocampus because hippocampal activity is known to be required for spatial learning and memory formation.
Results: Our behavioral analyses indicate elevated anxiety and deficits in learning and behavioral flexibility of a spatial memory task in the KO mouse. Consistent with the behavioral findings, KO mouse shows reduced brain mass and a significant decrease in the size of nuclei of the hippocampal pyramidal neurons along with impaired expression of the early phase of hippocampal long-term potentiation (LTP).
Discussion: Our study suggests that this TCblR/CD320 KO mouse that develops selective Cbl deficiency and functional deficits in the CNS could provide a model to understand the metabolic and genetic basis of neuro-pathologic changes due to Cbl deficiency.
Conflicts of Interest: None.

O39 Babak Hooshmand Vitamin B12, folate, and sulfur amino-acids as risk factors for dementia and cognitive decline: a longitudinal population based study
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Introduction: The association of vitamin B12, folate, and homocysteine (tHcy) with cognitive decline which precedes clinical dementia is controversial. Furthermore, the role of sulfur amino-acids other than tHcy on cognition has rarely been investigated.

Aim: To investigate the association of vitamin B12, folate, and sulfur amino-acids with the risk of incident dementia and the rate of cognitive decline in a longitudinal population-based study of Swedish older adults.

Methods: From the Swedish National Study of Aging and Care (SNAC-K), 2647 dementia-free subjects at baseline aged 60-102 years with repeated measures of Mini-mental state examination (MMSE) at 2-3 occasions over 6 years were recruited. Incident dementia was diagnosed in 202 persons. Linear mixed models and proportional hazards regression models were used to examine the relationships with cognitive decline and incident dementia, respectively.

Results: The adjusted hazard ratio (95% confidence interval (CI)) of dementia for the highest tHcy quartile compared to the lowest was 1.69 (1.01–2.82); p=0.046. In contrast, elevated methionine values were related to decreased dementia risk: hazard ratio (95% CI) was 0.62 (0.39–0.99); p=0.047 for the highest quartile of methionine. Compared with the lowest quartile, the highest tHcy and cysteine quartiles were related to faster MMSE decline over 6 years: β coefficient and standard error (SE) were -0.093 (0.04); P=0.011 for homocysteine and -0.100 (0.037); P=0.008 for cysteine. In contrast, higher methionine values tended to be associated with slower decline in MMSE score: β coefficient (SE) were 0.058 (0.032); P=0.073 for the top methionine quartile. No longitudinal associations with MMSE or incident dementia were found for B12, HoloTC or folate.

Discussion: Sulfur amino-acids may be related to incident dementia and more rapid cognitive decline over 6 years. Randomized clinical trials are needed to determine the impact of optimizing the levels of homocysteine, cysteine, and methionine on cognitive decline.

Conflicts of interest: ADS & HR hold consultancies with E. Merck

O40 Vincent Th. Ramaekers Folate receptor alpha autoimmunity in low-functioning autism and response to folinic acid treatment

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Introduction: We found a high prevalence of serum folate receptor (FRα) autoantibodies of the blocking type among children with low-functioning autism and their parents. An increased risk for mothers to have a child with autism could be attributed to prenatal folate deficiency. Reports suggesting a central role of oxidative stress among children with autism could be linked to instability of reduced folates and diminished cellular folate incorporation.

Aim and methods: Patients with low-functioning autism and their parents (76 families) have been screened for serum FRα autoantibodies and anti-oxidant parameters. Clinical assessment included the Childhood Autism Rating Scale (CARS). Treatment aimed to correct anti-oxidant deficiencies and upon finding FRα antibodies, high-dose folinic acid supplement (0.5-2 mg/kg/day) was added. Periodic clinical assessment was performed over two years.

Results: The mean age of patients was 4.4±2.3 years with a male/female ratio of 64:11. The prevalence of blocking and/or binding FRα autoantibodies in the autistic children and parents was 75% and 28% respectively, compared to 3.3 % in controls. Children with low-functioning autism had significantly elevated blood levels for oxidative DNA damage, increased copper/zinc ratio and compensatory increased ceruloplasmin, superoxide dismutase and protein thiols, whereas vitamin C levels were diminished. Correction of antioxidant deficiencies combined with high dose folinic acid resulted in significant improvement of autistic core signs (CARS at baseline mean±SD: 41.5±6.8 versus 34.6±6.3 following treatment (p<0.0001). Autism symptoms could be fully reversed in 17/76 children. Prognosis became less favorable as the child’s age increased beyond 6 years, if FRα antibody titers were high, in the event of maternal antibodies or positive antibodies in the child and both parents.

Discussion: FRα antibodies in 75% of children with autism and/or positive antibodies in either one or both parents (28%), represent the most frequent neurobiological etiology, amenable to treatment with significant overall improvement, despite the presence of adverse prognostic factors.
O41  Teodoro Bottiglieri  Plasma and cerebrospinal fluid biomarkers of the methylation cycle in cognitively normal and mild dementia of the Alzheimer type.

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Objectives: Substantial evidence implicates hypomethylation in the etiology and pathogenesis of Alzheimer’s disease (AD). In murine models DNA hypomethylation can regulate PS1 gene activity. Hypomethylation of protein phosphatase 2A leads to increase formation of phosphorylated Tau in brain tissue. Furthermore, homocysteine, a key metabolite in the methylation cycle is a risk factor for vascular disease and AD. We have undertaken a study to determine intermediates of the methylation cycle in plasma and CSF from a group of cognitively normal elderly subjects and those with mild AD, and examined the relationship with CSF Tau and β-amyloid40 (Aβ40) levels.

Methods: Plasma and CSF was collected from participants enrolled in longitudinal studies of healthy aging and dementia at the Knight Alzheimer’s Disease Research Center. The study included 92 individuals with a CDR of 0 (no dementia), 26 with a CDR of 0.5 (very mild dementia), and 13 with a CDR of 1 (mild dementia). LC-MS/MS was used to determine: 5-methyltetrahydrofolate (5-MTHF), total homocysteine (tHcy), methionine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), betaine, choline and cystathionine.

Results: In plasma significantly lower SAM and higher SAH concentrations were found in the CDR 1 group. In CSF significantly higher tHcy, cystathionine, betaine and choline were found in the CDR 1 group. CSF tHcy, SAH, betaine and choline were positively correlated with Tau, P-Tau181 and Aβ40 levels. 5-MTHF and the SAM/SAH ratio were negatively correlated with Tau, P-Tau181 and Aβ40 levels.

Conclusions: The associations between methylation metabolites, Tau and Aβ proteins provide further evidence for a role of these biomarkers in AD.

Conflicts of interest: The authors declare no conflicts of interest exist.

SESSION 12: B vitamins and cancer

O42  Abee L. Boyles  Challenges to evaluating evidence of risks from high intakes of folic acid

Abee L. Boyles.
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In May 2015, the National Toxicology Program held an expert panel meeting tasked with considering the available scientific literature and making research recommendations for evaluating the potential for adverse effects of high intake of folic acid. The panel considered the published literature within four general potential folate-related adverse health effect categories: cancer, cognition in conjunction with vitamin B12 deficiency, hypersensitivity-related outcomes, and thyroid and diabetes related disorders. Challenges faced when reviewing this literature included:

- No clear criteria to define a value for “high” intake
- Data gaps, as published research predominantly focused on risks from too little folate
• Varied methods of assessing folate exposure status including intake estimates reported in non-standardized units as well as biomarkers measured in serum, whole blood, and RBC using varied assays (HPLC-MS/MS, microbiological assay, or radioassay)
• Little consideration of other factors influencing the folate pool such as differing bioavailabilities among consumed folate species, folate consumption conditions, genetic variation in folate metabolism genes, and lifestyle factors.

The expert panel recommended developing better methods including measuring both intake and blood levels of total folate and specific forms, more assay standardization practices, and improved reliability of food and supplement composition tables.

O43 Despoina Theofylaktopoulou Impaired functional vitamin B6 status is associated with increased risk of lung cancer in the Lung Cancer Cohort Consortium (LC3)
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Introduction: Circulating vitamin B6 levels have been inversely associated with lung cancer. To date, most studies are limited to measurements of the vitamer pyridoxal 5'-phosphate (PLP) in serum/plasma.

Aim: We evaluated the prospective associations of the functional marker of B6 status, circulating 3-hydroxykynurenine:xanthurenic acid ratio (HK:XA), with risk of lung cancer in a nested case-control study.

Methods: The study included 5,364 incident lung cancer cases and 5,364 controls from 20 prospective cohorts. Metabolites were measured in a central laboratory (Bevital, www.bevital.no) using liquid chromatography–tandem mass spectrometry. We used conditional logistic regression to evaluate associations between HK:XA and lung cancer, and a random effect models to combine results from different cohorts.

Results: High levels of HK:XA, indicating impaired functional B6 status, were associated with an overall 22% increased risk of lung cancer, odds ratio (95% confidence interval) 1.22 (1.10, 1.35), for third vs. first tertile. However, there was substantial heterogeneity between cohorts that could be related to differences in sex distribution and smoking prevalence. In stratified analysis the risk of lung cancer was higher (approximately 50%) for those in the highest category of HK:XA in men, but not in women, and in former and current smokers, but not in never smokers. In analysis stratified by histology HK:XA was mainly associated with an increased risk of squamous cell carcinoma OR (95% CI) of 1.41 (1.10, 1.81), for third vs. first tertile, but not other histological types. The association for squamous cell carcinoma was consistent in analysis stratified by sex and smoking status.

Discussion: Our findings support that impaired functional B6 status may be involved in the pathogenesis of lung cancer, especially in squamous cell carcinoma.

Conflicts of interest: None to declare

Figure A. Forestplot showing odds ratios for lung cancer comparing the third to the first tertile of HK:XA. Conditional logistic regression was performed for each cohort and was adjusted for smoking intensity using quartiles of cotinine among smokers. Cases and controls were matched on age, sex, and smoking status. Results were combined using random effect models for each region and all studies combined.

B. Distribution of sex and smoking status in the different cohorts. ATBC, The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CLUE,
The Campaign Against Cancer and Stroke (CLUE I) and the Campaign Against Cancer and Heart Disease (CLUE II); CPS-II, The American Cancer Society Cancer Prevention Study-II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; HUNT, The Nord-Trøndelag Health Study; MCCS, The Melbourne Collaborative Cohort Study; MDCS, The Malmö Diet and Cancer Study; MEC, The Multiethnic Cohort; NHS, The Nurses’ Health Study; NSHDS, The Northern Sweden Health and Disease Study Cohort; NYUWHS, The New York University Women’s Health Study; PHS, Physicians’ Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SCCS, The Southern Community Cohort Study; SCS, The Shanghai Cohort Study; SMHS, The Shanghai Men’s Health Study; SWHS, The Shanghai

O4 Racha Zgheib Why Cancer Stem Cells are methionine dependent?
INSERM UMR 954 NGERE, 54000 Vandoeuvre, France.*equal contribution

Introduction: Some cancers require methionine to grow. The mechanisms of this methionine dependence are unknown. Cancers are composed of heterogeneous cell populations among which Tumor Initiating Cells (also termed Cancer Stem Cells) represent a small subset capable of self-renewal and pluripotency. Their eradication is clinically relevant because they escape treatment and cause later regression. Under anchorage-independent conditions they form spheroid floating structures known as tumor-spheres whereas the bulk cancer cells (monolayer cells) undergo cell death.

Aim: Identify the mechanisms of methionine dependency in cancer cells

Methods: Tumor-spheres were derived from a glioblastoma cell line, U251, and their activity was compared to the monolayer cells by quantitative real-time PCR and Immunofluorescence. RNA-seq followed by bioinformatics was performed to detect clusters of differential gene expression between Tumor spheres and monolayers cells at transcript level and functionally annotate them.

Results: Unlike monolayers cells, tumor-spheres are methionine dependent. There stem-like character was confirmed by the overexpression of the pluripotency markers SOX2, Nanog and OCT4. In addition, they overexpress the methylation relevant enzymes Carm1 and Prmt1, which are Arginine Methyltransferase Proteins, as well as methionine cycle enzymes like Methionine Synthase and Methionine Synthase Reductase (MTR and MTRR). Interestingly, Sox2, Nanog and OCT4 colocalize with MTR and MTRR in tumor spheres but not in monolayers cells. RNA-Seq analysis revealed the differences between tumor-spheres and monolayer cells in their expression of diverse transcriptional programs related to cell cycle, GTP/GDP binding, oncogenic signaling, and other cell processes like hormone or lipid metabolisms. Similarly, a neural stemness signature was derived from a consensus set of genes overexpressed in tumor spheres but not in monolayer cells.

Discussion: Transcriptional responses specific to tumor spheres were revealed. Further investigations are under way in order to confirm that they contribute to explain cancer cells methionine dependency.

The authors report no conflicts of interest in this work.

O45 Natalia I. Metabolic changes in GNMT knockout mice during progression from Krupenko steatosis to hepatocellular carcinoma.
Natalia I. Krupenko.
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Introduction: Glycine N-methyltransferase (GNMT), a major liver enzyme, catalyzes the transfer of methyl group from S-adenosylmethionine (SAM) to glycine, producing sarcosine and S-adenosylhomocysteine. This reaction is regulated by 5-methyltetrahydrofolate, which binds to the protein and inhibits its enzymatic activity. These attributes position GNMT as a master regulator of cellular methylation. Indeed, the GNMT knockout mice demonstrate drastic elevation of SAM and altered genome methylation. In our experiments, these mice developed fatty livers at about three months of age, and hepatocellular carcinomas by the age of 6 months. Untargeted metabolomics
profiling of the 3-month old Gnmt-/- animals showed dramatic elevation of liver SAM (more than 400-fold) as well as significant alterations in numerous metabolites. Out of 538 metabolites analyzed, 99 were decreased more than two-fold, and 61 were elevated more than twice in the knockout livers, indicating strong metabolic re-programming.

**Aim:** In the present study, we have analyzed metabolomic profiles of the livers of 4.5 month-old GNMT-/- and wild type mice, targeting the question: what changes in the metabolic profiles occur during transition from steatosis to the pre-cancerous/early malignant state.

**Methods:** A global untargeted metabolomic profiling of livers from the 4.5 month old mice was performed using a Metabolon® platform.

**Results:** The comparison of metabolites in GNMT KO vs. WT mice at 4.5 months and at 3 months of age indicated that changes in 28 metabolites were reversed at 4.5 months while numerous metabolites (more than 20) continued to further deviate from the "normal" levels. Among the latter, most prominent alterations were observed for SAM; 5-methylthioadenosine (indicator of polyamine synthesis); dihydroacetone phosphate (likely indicator of glycolysis/Warburg effect); derivatives of taurine, and fatty acid metabolites.

**Discussion:** We suggest that metabolic patterns associated with these two groups of metabolites (whether they are markers or drivers of tumorigenesis) might help to define pathways associated with progression from steatosis to malignant lesions.

**Conflict of interest:** The author declares no conflict of interest.

**SESSION 13: Bioavailability of B vitamins**

O46 Jette Jakobsen Principle for studying the potency of the different vitamin D active compounds – usable in the vitamin B community?

Jette Jakobsen.
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Vitamin D, the sunshine vitamin, derived primarily from the production in vertebrate skin, where it is converted from 7-dehydrocholesterol by UV-B from the sun. In food vitamin D activity is possessed by cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), and the corresponding hydroxylated forms 25-hydroxycholecalciferol (25OHD3) and 25-hydroxyvitamin D2.

Vitamin D3 is the major vitamer in food, while the distribution of vitamin D2 is primarily in wild mushrooms and UVB exposed mushrooms, and in minor amounts in animal products, due to production from UVB exposure of ergosterol in e.g. plants. 25-hydroxy vitamin D3 and 25-hydroxyvitamin D2 (25OHD) is the compounds produced in vertebrates by 25-hydroxylation of vitamin D3 and vitamin D2 (vitamin D), and therefore present in food of animal origin.

Vitamin D is produced throughout the biological kingdom and functions mainly in gene transcription. The accepted biomarker for vitamin D status is the content of 25-hydroxyvitamin D in plasma or serum. The level for sufficient vitamin D status is discussed around the world, but between 50-80 nmol/L. Similar for the recommended dietary intake of vitamin D is challenged, and has raised from 5 μg per day to 15 μg per day in the last decade, depending on the region in the world.

Although vitamin D3 and vitamin D2 have been considered to have the same potency since the 1940’s, this consensus has been challenged for the last 20 years. Randomized control trials (RCT) using vitamin D status as endpoint for supplementation with vitamin D3 and vitamin D2 has shown conflicting results. The reason for this seems partly to be because vitamin D2 upregulates 24-hydroxylation, leading to increased metabolic degradation of the 25OHD2.

No consensus is present for the potency of 25OHD3 compared to vitamin D3, and studies using RCT with vitamin D status as endpoint do similar as for vitamin D2 and vitamin D3 give conflicting results.

Most of the studies conducted aimed to assess the supplementation to eradicate deficiency, while few included levels consumed in the diet for healthy subjects. Furthermore, the reason for the confliction results seems to be the different in design e.g. vitamin D status at start, length of study, but also the very import variable in the vitamin D community, namely the analytical assay used.

From food, the absorption of vitamin D active compounds depends on a list of factors, which are
considered as “SLEMENGHI” - Species (vitamer), Linkage (bound forms), Amount (in the meal), Matrix (fatty/nonfatty), Nutrient (Vitamin D status in the subject), Host-related factors (genes), Interactions (between the variables).

These factors will found the basis for the talk, which will focus on the methods used to assess the difference in potency between the vitamin D vitamers.

O47 Sergey N. Fedosov

B12 in milk, its protein complexes and bioavailability


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Introduction. Milk is an important source of cobalamin (Cbl, vitamin B12), but its protein association patterns remain largely unexplored. Some Cbl-binding proteins were earlier detected in the dietary milk, e.g. transcobalamin (TC) or haptocorrin (HC) in bovine or human samples, respectively. Binding to both proteins is extremely strong but TC liberates Cbl at pH2, which facilitates its intestinal uptake.

Aim. We examine Cbl-related proteins in bulk milk from cows (Denmark, India) and water buffalo (Italy, India).

Methods. Gel-filtration separated the main milk proteins. The endogenous Cbl and the binding of CNCbl/HOCbl were tested. Affinity purification on Sepharose-Cbl was used.

Results. The total endogenous Cbl was comparable in all samples (1.5-3 nM) but its distribution and protein binding profiles varied (Table). TC-Cbl was omnipresent – highest in cows, lowest in buffalo. HC-Cbl was low in cows but high in buffalo. Only buffalo milk bound added CNCbl to the unsaturated TC (India, Italy) and the excessive HC (Italy). Buffalo HC was purified and characterized. The unexpected binding pattern was observed for casein fraction that coordinated high quantities of HOHbl (>3 mM) via His/HO-substitution (Kd = 0.1 mM) specific for HOHbl. The endogenous casein-HisCbl complexes were observed only in bovine milk, where Cbl exceeded the binding capacity of TC(HC). Caseins also contained small quantities of the entrapped TC(HC). Transfer of Cbl to human intestinal proteins was easy for TC-Cbl (pH sensitivity, fast proteolysis) but considerably hampered for buffalo HC-Cbl (pH stability, slow proteolysis). Transfer of Cbl from casein/peptides required 2-3 h at the physiological concentrations disregarding pH.

Conclusions. Availability of Cbl is expected to be high for milk containing TC-Cbl (cow breeds) but potentially constraint for the samples with HC-Cbl, especially at high unsaturated HC (buffalo, Italy). The role of casein/peptides in relation to the Cbl-uptake remains unsettled.

Table. The Cbl-binding proteins of milk, their endogenous Cbl and the specific binding capacity.

<table>
<thead>
<tr>
<th>Milk</th>
<th>casein-Cbl nM</th>
<th>HC-Cbl nM</th>
<th>TC-Cbl nM</th>
<th>casein nM</th>
<th>HC nM</th>
<th>TC nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>cow, DK</td>
<td>1.5</td>
<td>&lt; 0.1</td>
<td>1.3</td>
<td>&lt; 0.1, CNCbl</td>
<td>0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>cow, IN</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>&lt; 0.1, CNCbl</td>
<td>0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>buffalo, IN</td>
<td>0.7 (TC/HC)</td>
<td>1.7</td>
<td>0.3</td>
<td>≈ 0.3, CNCbl</td>
<td>&lt; 0.1</td>
<td>2.6</td>
</tr>
<tr>
<td>buffalo, IT</td>
<td>≈ 0</td>
<td>1.7</td>
<td>0.2</td>
<td>&lt; 0.1, CNCbl</td>
<td>23</td>
<td>4</td>
</tr>
</tbody>
</table>

O48 Klaus Pietrzik

Development and validation of the concept of personalized folate supplementation to achieve protective RBC-folate concentrations in young women

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Introduction: Evidence-based recommendations are needed to achieve desirable pre-pregnancy concentrations of red blood cell (RBC)-folate.

Aim: To develop personalized folate supplementation models based on the response of RBC-folate to a given dose and duration of supplementation.

Methods: The time- and dose-dependent changes of RBC-folate concentrations were modelled following 400 or 800 μg/d folate administered for 8 weeks in a randomized open-labelled controlled trial in 172 women. Dose- and time-dependent RBC-folate loading capacities were calculated and used to predict post-treatment RBC-folate. In a subgroup of 119 women, the individually predicted RBC-folate concentrations were compared with the measured ones between weeks 4 and 8.

Results: The median change of RBC-folate from baseline was higher in the 800 than in the 400 μg/d group (275 vs. 169 nmol/L after 4 weeks, and 552 vs. 346 nmol/L after 8 weeks; p < 0.001). The median RBC-loading capacities [= measured RBC-folate – (baseline RBC-folate * RBC-survival rate)] was (299 vs. 409 nmol/L) at 4 weeks and (630 vs. 795 nmol/L) at 8 weeks in the 400 and 800 μg/d, respectively. After applying Deming regression and recalibration, the predicted post-treatment RBC-folate concentrations = 25 + 1.27 * baseline RBC-folate (4 weeks, 400 μg/d) or = 65 +1.41 * baseline RBC-folate (4 weeks, 800 μg/d) did not differ from the measured concentrations. The predicted and the measured RBC-folate concentrations showed high agreement in the validation cohort between weeks 4 and 8.

Discussion: The suggested models can guide personalized supplementation of folate in women when baseline RBC-folate concentrations are measured and the time to pregnancy is 4 to 8 weeks. The trial was registered at The German Clinical Trials Register: DRKS-ID: DRKS00009770.

Conflict of interest: The authors have no conflict of interest. BioTeSys GmbH is an independent third party research institute that was responsible for designing the study, recruitment, performance, data collection, measurements and data analyses. The original trial was initiated and sponsored by Merck Selbstandikation GmbH. The sponsor had no role in conducting the study, the concept of the present publication, data analysis, or reporting the results.

049 Christian B. Juul Bovine transcobalamin enhances in vitro intestinal absorption of cobalamin in a receptor-associated protein sensitive manner

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Introduction: Deficiency of cobalamin (Cbl, vitamin B12) is associated with several and severe clinical manifestations. Cbl is acquired by consumption of animal source foods, and cow’s milk has been identified as an excellent source of Cbl in the form of hydroxocobalamin (HOCbl). Half of the HOCbl in cow’s milk is in complex with the Cbl-carrying protein, transcobalamin (TC). In the blood TC-Cbl is the active fraction of Cbl and a ligand for the receptors CD320 and megalin.

Aim: To gain insights into the effect of bovine TC (bTC) on intestinal absorption of Cbl and the underlying mechanisms.

Methods: Differentiated and polarized Caco-2 cells were used in a two-compartment system as a model of the human small intestine. Effects of recombinant bovine TC (rbTC) and receptor-associated protein (RAP, a potent antagonist of megalin binding) on absorption of HOCbl were investigated. The presence of CD320 and megalin was examined by immunocytochemical staining of the enterocyte-like Caco-2 cells.

Results: The cellular transcytosis and accumulation of HOCbl in complex with rbTC increased respectively 60 and 30-fold compared to the free HOCbl. Inclusion of RAP was accompanied by a marked concentration-dependent decrease in the cellular accumulation and transcytosis of HOCbl. Immunocytochemical staining of Caco-2 cells were positive for both CD320 and megalin.
Discussion: The ability of rbTC to enhance the in vitro absorption of Cbl in the Caco-2 model indicates that bTC potentially contributes to the bioavailability of HOCbl in cow’s milk. Absorption of rbTC-HOCbl in the Caco-2 cells is likely governed by the RAP-sensitive receptor megalin, as CD320 is reported being insensitive to RAP. The stability of cow’s milk bTC-HOCbl in the human digestive tract is not known, but the complex would likely be subject to digestion. It could be speculated that targeted delivery of bTC-Cbl at the site of absorption could be used in treating Cbl deficiency.

Conflicts of interest: None to declare

O50 Eamon Laird Vitamin B12 from dairy sources: potential benefits for bone health; Findings from the TUDA Study.

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Osteoporosis is a common, chronic condition with 6% of men and 21% of women aged 50-84yrs affected in the EU (1). It is characterized by decreased bone mineral density (BMD) with increased risk of fracture (2). Studies have observed positive associations between dairy intakes and BMD (3). Vitamin B12 is only available from relatively few animal sources and among these there is some evidence that milk and dairy products may provide a superior source (4) and could contribute to the positive effects of dairy on bone health (5) though few studies have examined this. The Trinity Ulster Department of Agriculture (TUDA) ageing cohort study is a large study of older Irish adults (>60 yrs; n 5186), designed to investigate nutritional factors, gene-nutrient interactions and health/lifestyle factors in the development of chronic diseases of ageing. Blood samples, DXA scans and a FFQ were examined to examine biomarkers of B12, intakes of dairy foods and bone health status. Participants with the highest daily intakes of milk and yogurt (ounce per day intake serving) compared to the lowest intakes (1-2 times per week/less) had significantly higher total cobalamin concentrations (303 vs 264 pmol/L respectfully). Furthermore, each unit increase in daily yogurt intake resulted in an increase of 24.3 pmol/L of total cobalamin concentrations after adjustment for covariates. Additionally, total hip and femoral neck BMD were 3.1 - 3.9 % higher among those with the highest yogurt intakes compared to the lowest after adjustment (P<0.05).

Our observational findings cannot give definitive evidence of a contributory role for vitamin B12 in bone health. However, in these older adults, dairy foods are a rich source of vitamin B12 and the findings potentially indicate that vitamin B12, (probably through increased absorption from dairy foods (5)) may have positive benefits for bone health. Future RCT trials are required to investigate the efficacy of such approaches.


Acknowledgement of Funding: Funding to conduct this work was provided by the Irish Department
of Agriculture, Food and the Marine through the grants 07FHRIUCD1 ("JINGO" 2007-2013) and 13F407 ("JINGO-JPI"/"ENPADASI" 2014-2016) and from the Northern Ireland Department for Employment and Learning (DEL), Cross-Border Research and Development Programme: “Strengthening the all Island Research Base”. Funding was also provided from The National Dairy Council (NDC) Ireland.

SESSION 14: Betaine and Choline – the link to B vitamins and homocysteine

O5  Steven H. Zeisel  How do perturbations in choline metabolism cause altered gene expression?

Steven H. Zeisel. University of North Carolina, Nutrition Research Institute, Department of Nutrition, Kannapolis, NC 28081.

We are actively investigating how perturbations in choline metabolism cause altered gene expression that then results in changes in brain development and in carcinogenesis in the liver. If maternal intake of choline during pregnancy is low, epidermal growth factor receptor (EGFR) signaling in fetal brain cortical neural progenitor cells is disrupted (Egfr mRNA is made but not translated into protein), leading to a reduction in the numbers of upper layer cortical neurons produced during mid- and late gestation. We found that upregulation of microRNA-129-5p (miR-129-5p) in response to reduced maternal intake of choline underlies aberrant EGFR protein synthesis in cortical neural progenitor cells. Betaine-homocysteine S-methyltransferase (Bhmt)-null mice develop spontaneous cancers of the liver, and in their livers compared to WT, S-adenosylhomocysteine concentrations are elevated, changing methylation potential. We identified 63 differentially methylated CpGs (proximal to 81 genes (across 14 chromosomes), 18 of which were differentially expressed. We found a hypomethylated region mapping to IQ motif-containing GTPase activating protein 2 (Iqgap2) and Proteinase-Activated Receptor-3 (F2rl2), two genes that were also silenced and under-expressed in Bhmt-null mice. These two genes are known to be tumor suppressor genes. We are studying the involvement of microRNAs in these changes in gene expression. We are also studying similar mechanisms involved in adipocyte browning in Bhmt-null mice. We propose that the effects of perturbing choline metabolism are mediated by changes in epigenetic marks which in turn change the expression of specific microRNAs, that in turn alter expression of critical proteins in a tissue specific manner.

This work was supported, in part by a grant from the National Institutes of Health (DK56350). Dr. Zeisel serves on scientific advisory boards for Metabolon and SNPitty and has an equity interest in Nutrigene Sciences. He consults for the Campaign for Essential Nutrients (CFEN; supported by Bayer Consumer Healthcare, DSM and Pharmavite).

O52  Anne Bozack  Results from a folic acid and creatine supplementation trial in Bangladesh: Differences in treatment effect on arsenic methylation, blood arsenic, and homocysteine by baseline choline and betaine status


Department of Environmental Health Sciences, Columbia University (AB, AMLL, VI, FP, JHG, MVG); Department of Epidemiology, Columbia University (MNH), Department of Biostatistics, Columbia University (XL), Columbia University Arsenic Project in Bangladesh, Dhaka, Bangladesh (ABS, HS, TI).

Introduction: Exposure to arsenic (As), a human carcinogen, persists in many regions of the world. Inorganic As (iAs) undergoes methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which is more readily excreted through urine. Folic acid (FA) supplementation has been demonstrated to increase the proportion of urinary DMA and to lower blood As (bAs). Arsenic methylation patterns are also associated with urinary creatinine, possibly due to the consumption of methyl donors for creatine biosynthesis. Arsenic methylation may be impacted by other nutritional factors involved in one-carbon metabolism.

Aim: Our aim was to determine the effects of 400 and 800 μg/day FA and/or 3 g/day creatine
supplementation on the proportion of urinary As metabolites, bAs, and homocysteine stratified by baseline choline and betaine.

**Methods:** We conducted a randomized controlled trial in Bangladesh. Participants were randomized to receive 400 μg FA, 800 μg FA, 3 g creatine, 400 μg FA + 3 g creatine, or placebo.

**Results:** Overall, there was a trend toward greater treatment effects among participants having low choline and/or betaine (i.e., below the median). The effects of FA and creatine supplementation at 12 weeks differed by baseline choline and betaine status; in adjusted models for change in percent urinary As metabolites and bAs, there was a significant interaction between treatment and baseline choline and betaine status (p<0.05). A significant treatment effect of creatine on change in urinary %MMA and homocysteine was observed among participants with low baseline choline and betaine levels (p<0.05), but not among participants with levels above the median or the full treatment group.

**Discussion:** Arsenic methylation capacity is associated with the availability of methyl donors. The extent to which FA and creatine supplementation augment As methylation capacity is influenced by choline and betaine, nutritional factors involved in one-carbon metabolism.

**Conflicts of interest:** None

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**O53** Maria F. Mujica-Coopman

**Serum betaine and total vitamin B-12, but not folate, concentrations are negative predictors of total homocysteine concentration in Canadian pregnant women of South Asian and Caucasian ethnicity**

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*shared first authorship.

**Introduction:** Betaine, a metabolite of choline, is an important methyl donor in the folate-vitamin B-12 independent remethylation of homocysteine. South Asian populations are vulnerable to an impaired one-carbon metabolism due to a higher risk of B-12 deficiency. To date, betaine concentration and its association with other methyl donors have not been described in South Asian pregnant women.

**Aim:** To determine betaine concentrations of pregnant women of South Asian and, for comparison, Caucasian ethnicity and investigate its relationship with total B-12, folate, and total homocysteine (tHcy) concentrations in 1st and 2nd trimester of pregnancy.

**Methods:** This retrospective cohort study included 748 apparently healthy pregnant women of South Asian (50%) and Caucasian ethnicity from Vancouver, Canada. Biomarkers were quantified in non-fasting maternal serum samples collected at 9–13 and 15–19 gestational weeks. The interdependency of one-carbon metabolites was determined using mixed effects models, adjusted for repeated measures, on natural log-transformed data.

**Results:** Mean (95%CI) concentration of serum betaine (μmol/L) was significantly higher in South Asian pregnant women in 1st and 2nd trimester [21.1 (20.2; 21.9) and 16.7 (16.1; 17.4), respectively] compared to that of Caucasian women [18.6 (17.9; 19.4) and 13.7 (13.2; 14.3), respectively] (Wilcoxon rank-sum test; P<0.0001). Maternal betaine concentration was positively predicted by maternal serum total B-12 (P=0.04), folate (P=0.01), and choline (P=0.001) concentrations and negatively by South Asian ethnicity (P<0.001). Maternal serum total B-12 and betaine, but not folate, concentrations were negative predictors (P=0.02 and P<0.0001) of maternal tHcy concentration in this population with high folate status [e.g., 1st trimester mean (95% CI) serum folate: 65.6 (63.7; 67.7) nmol/L].

**Discussion:** South Asian pregnant women had a significantly higher betaine status. The association between betaine and B-12, but not folate, with tHcy status suggests that in a folate-replete population circulating tHcy concentrations may be determined by betaine and B-12 rather than folate status.

**Conflict of Interest:** none to declare.
A Systematic Review and Meta-analysis on the efficacy of Betaine in the treatment of Cystathione B-synthase deficient patients who are non-responsive to pyridoxine

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Classical Homocystinuria is an inborn error of metabolism (IEM) due to the deficient activity of cystathionine beta-synthase, which causes ocular, skeletal, neurological and vascular disease (thromboembolism). Therapy includes low-methionine diet, pyridoxine, folic acid and vitamin B12 to improve the clinical manifestations, in special the cardiovascular risk. An alternative strategy for treatment is betaine, a drug which converts homocysteine to methionine.

Aim: to estimate the pooled efficacy of Betaine for Classical Homocystinuria in pyridoxine-unresponsive patients.

Methods: Systematic Review and Meta-analysis. The following databases were searched: MEDLINE/PubMed, EMBASE, LILACS, SCOPUS and the Cochrane Library. The search was limited to clinical trials published until January 4th, 2016. The systematic review was conducted according to the method proposed by the Cochrane Collaboration. Plasma total free homocysteine (tHcy) was analysed as a surrogate outcome. The mean tHcy decline associated to the use of Betaine was summarized with random effects model, using R software version 3.3.5 and meta package version 4.4-1.

Results: We screened 429 papers after exclusion of duplicate results and reviewed 71 abstracts and full texts of interest. Four papers were included in the meta-analysis, being only one a randomized controlled trial. Betaine was prescribed considering body weight (from 0.02 to 0.25g/kg/day) or in a fixed dose of 6g/day across included studies. tHcy was the most frequent outcome evaluated. The pooled mean of decline of tHcy after the use of Betaine was -44.24% (95%CI = -83.07; -5.40), with high heterogeneity (I-square 86.9%) (Figure 1). Random-effects model could have reduced this impact.

Conclusion: Even with small data volume and with heterogeneous results, we could generate pooled estimates supporting the Betaine use for CBS deficient, pyridoxine-unresponsive patients.

Figure 1. Cystathionine Beta-Synthase Deficiency - Meta-analysis of Mean total free homocysteine decline after Betaine treatment including only Piridoxine nonresponsive patients.

The Effects of Betaine on Brain and Liver in Methionine & Choline Deficient Rats

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**Introduction:** Abnormal liver metabolism might limit the brain’s supply of methyl-donors, thereby contributing to neurodegenerative disease. Dietary deficiency of the methyl-donors Methionine and Choline (MCD) is a well-established model of non-alcoholic fatty liver disease (NAFLD), yet neurologic outcomes have not been studied in this model. Theoretically, dietary betaine could partly compensate for impaired methylation in this model, by conserving methionine and choline via betaine-homocysteine methyltransferase (BHMT). Because BHMT occurs predominantly in liver but not in brain, we hypothesized that neuroprotective effects of betaine would be attributable to liver metabolism.

**Aim:** To characterize the metabolic effects of the MCD diet in liver and brain with and without supplemental betaine.

**Methods:** Male SD rats were fed a Control, MCD or betaine supplemented MCD diet (MCD+B) for 8 weeks before collecting blood, liver and brain.

**Results:** Betaine prevented MCD-induced NAFLD. Despite this, plasma homocysteine was higher in MCD+B rats compared to the MCD and controls (23.1±7.2, vs. 13.1±10.1 and 8.5±5.2 μmol/L respectively; p<0.01). MCD+B suppressed and MCD increased liver methylation potential (SAM/SAH ratio) compared to controls (1.6 and 0.2 vs. 0.7 respectively; p<0.0001). In contrast, brain methylation potential resisted dietary changes (SAM/SAH=10.2, 11.2 & 10 74 ++ .8 for MCD+B, MCD and controls, respectively). Although neither methionine nor choline differed significantly by diet in liver and brain, liver methionine and brain choline were ~20% lower on average in MCD rats than in controls, and this was normalized by betaine. Interestingly, brain betaine was 22-times higher in MCD+B than in MCD fed rats and 5-times higher than in controls ( 85.8 vs. 3.8 and 16.4 respectively; P<0.0001).

**Discussion:** Betaine is hepato-protective in the MCD-model of NAFLD. Because the brain lacks BHMT, any neuroprotective effects of betaine are likely to follow from the amelioration of fatty liver or from direct effects of betaine in brain.

**SESSION 16: B vitamins and treatment**

O56 Eva Greibe Cyano-B12 versus hydroxo-B12: New insights on transport and accumulation

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Vitamin pills normally contain cyano-B12 (CN-B12), the synthetic form of vitamin B12; whereas food contains mainly hydroxo-B12 (HO-B12). In general, the two forms are considered of equal nutritional value; however, our recent data - and also the data of others – suggest that the two forms are handled differently in the body. In a clinical setting, we find that CN-B12 accumulates to a higher degree in the plasma than HO-B12. In a rat model, we find no difference in the uptake of orally administered free labeled CN-B12 and HO-B12; but we observe marked differences in the tissue distribution. Notable the liver accumulates two-three-fold more HO-B12 than CN-B12 measured 24 hours and seven days after the oral administration; while slightly more CN-B12 than HO-B12 is present in the brain. Together with a higher accumulation of CN-B12 in plasma, our data suggests that in general HO-B12 is internalized by the cells more efficiently than CN-B12. We explored the conversion of the administered B12 to the coenzyme forms of B12 after two weeks of treatment with CN-B12 and found the fraction remaining as CN-B12 to be 3% in the liver while it was as high as 20% in the brain. Finally, we explored the transfer of labeled CN-B12 and HO-B12 to “two days prior to birth” fetuses 24 hours after oral administration to the pregnant rat. An equal amount of the two forms of B12 was transferred to the fetus, but more CN-B12 than HO-B12 accumulated in the cephalic region. In conclusion, CN-B12 and HO-B12 are absorbed alike but show marked differences in tissue distribution, thereby questioning the equal value of different molecular forms of B12 for treatment and prevention of B12 deficiency.
Bacterial Folates Provide an Exogenous Signal for C. elegans Germline Stem Cell Proliferation

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Animal germ stem cells (GSCs) provide reproductive cells to allow species propagation. Caenorhabditis elegans, a hermaphrodite, feeds solely on bacteria, and its GSCs proliferate in adult stem cell niches located in the distal regions of the two gonad arms. We used a C. elegans mutant which produces intact non-syncytial GSC at a very high rate in vivo and capable of Edu incorporation in vitro. In this study, we observed that bacterial extracts promoted increased GSC proliferation both in vivo and in vitro. These data where further corroborated by the use of affinity chromatography for folate purification. Analysis of folate from three bacterial species by affinity/HPLC, revealed that they consist of derivatives with three or more glutamyl residues, the majority of which are made of (10)formylated-THF. We showed that stimulation of GSC proliferation is due solely to 10-formylTHF and 5,10-methenylTHF with stimulation increasing with increase in polyglutamation. This suggests 1) that transport of 10-formylTHF from the intestine to GSCs is without prior deconjugation or conversion to other forms and 2) concentration of 10-formylTHF(6) (1nM) for optimal GSC stimulation is much lower than that needed for one carbon metabolism. Consistent with this conclusion is the demonstration that in vivo and in vitro stimulation of GSC proliferation is also attained by dihydropteroate. We considered the possibility that stimulation of GSC proliferation is catalyzed by a folate receptor (FR). C. elegans contains an apparent ortholog of FR, folr-1 (C17G1.1), which has a predicted signal peptide and transmembrane domain that is compatible with cell surface localization. We showed that RNAi depletion of folr-1/FR abolishes the stimulatory effect of folate on GSC proliferation both in vivo and in vitro. These data strongly imply that the stimulation of GSC proliferation by bacterial folate is due to signaling rather than to canonical one carbon metabolism.

Vitamin B12 status and absorption before and after bariatric surgery: A prospective study

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Introduction: Bariatric surgery implies a risk of developing micronutrient deficiencies, including vitamin B12 (B12) deficiency. We analysed the changes in biomarkers of B12 status before and after bariatric surgery, i.e. plasma B12, holotranscobalamin (holoTC), and methylmalonic acid (MMA) as well as the B12 absorption capacity.

Patients and methods: Patients (n=27) who underwent either Roux-en-Y Gastric Bypass (n=19) or Sleeve Gastrectomy (n=8) were included (n=18 were females). Blood samples were drawn before and 2 and 6 month after surgery. The B12 absorption capacity was evaluated by holoTC measurement after two days of a standardised B12 loading dose.

Results: Plasma B12 remained unchanged during the first months but then declined significantly around 6 months following surgery. In contrast, we observed a significant decrease in holoTC and increase in MMA already during the first months after surgery. B12 absorption was undetectable in all but one patient after both RYGB and SG.

Conclusion: Following bariatric surgery, this representative patient cohort lost the ability to absorb vitamin B12 and entered a stage of negative B12 balance mirrored by changes in holoTC and MMA and subsequently B12. Our data strongly suggests that treatment with pharmacological doses of B12 is warranted immediately after surgery. In addition, our data underpin that B12 is inferior to holoTC and MMA to detect early changes in B12 status. Importantly, we question the concept that the liver B12 stores suffice for long-term maintenance of an unchanged B12 status.

Paraoxonase 1 protects against homocysteine-thiolactone accumulation in humans

Joanna Perła-Kajan
Introduction: Nutritional or genetic disorders in homocysteine (Hcy) and folate metabolism elevate Hcy-thiolactone and lead to cardiovascular disease (CVD). Hcy-thiolactone, generated in an error-editing reaction when Hcy is selected in place of methionine during protein biosynthesis, is cytotoxic in cell cultures and experimental animals, causes protein damage, and induces proatherogenic changes in gene expression in human vascular endothelial cells (Gurda et al., Amino Acids 2015;47(7):1319-39). A cardioprotective enzyme, paraoxonase 1 (PON1), carried on high-density lipoprotein in the circulation, has the ability to hydrolyze Hcy-thiolactone in vitro.

Aim: To determine PON1’s role in vivo in humans, we studied how the natural variation in its activity affects Hcy-thiolactone levels in CVD patients.

Methods: We quantified urinary Hcy-thiolactone by HPLC. Serum PON1 activity was assayed with paraoxon (POase) and phenylacetate (PhAcase) as substrates in a subset of random samples from the Western Norway B-Vitamin Intervention Trial (n=85, 20.5% female, mean age 61.7 years). We analyzed samples from participants treated with (1) folic acid, vitamin B12, vitamin B6 (n=20); (2) folic acid, vitamin B12 (n=24); (3) vitamin B6 (n=23); or (4) placebo (n=18). Linear regression was used to study relationships between POase or PhAcase and Hcy-thiolactone/creatinine.

Results: POase, but not PhAcase, was negatively correlated with Hcy-thiolactone/creatinine (n=85, r=-0.21, p<0.05). Hcy-thiolactone/creatinine was significantly reduced in the 3rd and 2nd tertiles vs. 1st tertile of POase values (Table). However, there were no significant differences in Hcy-thiolactone/creatinine between 1st, 2nd, and 3rd tertiles of PhAcase. We also found that POase activity and Hcy-thiolactone/creatinine values were not modified by B-vitamin treatment.

Discussion: POase activity, a surrogate of the natural Hcy-thiolactonase activity of PON1, is a negative determinant of Hcy-thiolactone levels in humans. Pharmacologic or dietary enhancement of PON1 expression/activity might provide basis of CVD treatment/prevention.

Conflicts of interest: The authors declare no conflict of interest.


O60 Irene Pusceddu The role of homocysteine and B vitamins in telomere length: results from the cross-sectional and interventional trials

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Background: Telomeres are essential for the maintenance of genomic integrity. Telomere length declines with age and telomere dysfunction has been proposed as a biomarker for age-related diseases. Vitamin B12, B6 and folic acid are essential cofactors for numerous cellular processes including the synthesis of purines and nucleotides, DNA and protein methylation. B vitamin deficiencies and hyperhomocysteinemia are risk factors for the development of age-related diseases. The aim of this study is to evaluate the effects of B vitamins on telomere biology.
**Methods:** We analyzed the LURIC study (3316 cardiovascular patients), the Sud-Tyrolean study (STVS, 350 healthy subjects) and the KN0VIB study (60 elderly subjects were supplemented for one year with either vitamin B12, B6, folate, vitamin D and calcium (group A n=31) or only with vitamin D and calcium (group B n=29)). Relative telomere length (RTL), LINE-1 methylation, vitamin B6, B9, B12, homocysteine (Hcy), 5-methyltetrahydrofolate (5-methylTHF), 5,10-methenylTHF, S-adenosylhomocysteine, S-adenosylmethionine (SAM), cystathionine, dimethylglycine, methylmalonic acid, choline, IL-6, C-reactive protein (CRP) and advanced glycation endproducts (AGEs) were quantified.

**Results:** Median Hcy was 9.8 μmol/L in the STVS and 12.4 μmol/L in the LURIC study. Agecorrected RTL correlated negatively with Hcy (r=-0.151; p=0.007). RTL was shorter in the presence of hyperhomocysteinemia. Hcy was also lower in the highest (4th) quartile of age-corrected RTL. In the LURIC study, age-corrected RTL correlated positively with vitamin B6 (r=0.04; p=0.031), and the 4th quartile of age-corrected RTL was characterized by higher levels of vitamin B6 and folic acid and lower levels of IL-6 and hsCRP. Age-corrected RTL correlated negatively with IL-6 (r=-0.043; p=0.019). IL-6 and hsCRP correlated negatively with vitamin B6, folic acid, and positively with Hcy. In the STVS age-corrected RTL correlated negatively with AGES (r=-0.146, p=0.01). AGES correlated positively with Hcy and negatively with vitamin B12. In fact, AGES were higher in subjects with vitamin B12 below the median. In the interventional study, at baseline Hcy and 5-methylTHF were significant predictors of RTL. Vitamins supplementation decreased Hcy in group A but not in group B. Vitamins supplementation in group A increased LINE-1-methylation but reduced it in group B. After supplementation in group B but not in group A LINE-1-methylation correlated inversely with RTL, and LINE-1-methylation variation was an independent predictor of RTL variations. In group B an increase in RTL was correlated with lower LINE-1-methylation. Subjects with 5-methylTHF >10nmol/L had compared with <10nmol/L at baseline lower LINE-1-methylation, due to a to a lower SAM formation. Subjects with Hcy >12μmol/L had compared <12μmol/L at baseline and after supplementation longer telomeres. In group B subjects with Hcy >12μmol/L had lower mean LINE-1-methylation. Multiple backward regression analysis showed, 5-methylTHF in group A and Hcy in group B were significant predictors for LINE-1-methylation.

**Conclusions:** The results from these studies provide evidence for an association between vitamin B6, B12, folic acid, HCY and telomere length. Hyperhomocysteinemia is able to negatively affect telomere length in healthy, in cardiovascular patients and in elderly. On one hand hyperhomocysteinemia is able to induce an inflammatory and oxidant status that in turn induces telomere attrition. On the other hand hyperhomocysteinemia induces DNA hypomethylation that in turn induces telomere dysfunction. In fact, literature data indicates that DNA hypomethylation is associated with elongated and dysfunctional telomeres. Further analyses are needed to confirm these results.

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**O61 Matthew Bonem Development of an Enzyme Therapeutic for Classical Homocystinuria**

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**Introduction:** Classical homocystinuria is caused by a genetic defect in the cystathionine-β-synthase gene. Disruption of this metabolic pathway results in hyperhomocysteinemia, a condition where serum levels of total homocysteine (tHcy) are severely elevated. Hallmarks of homocystinuria include problems with the skeletal, ocular, vascular, and nervous systems. These symptoms are likely caused by homocysteine accumulation in serum as well as intracellularly.

**Aim:** The aim of this work is to develop a novel, enzymatic therapeutic capable of degrading excess serum homocysteine and create a new treatment for homocystinuria. We are engineering the human cystathionine-γ-lyase (GCL) enzyme to degrade homocysteine. The efficacy of preliminary enzymes has been tested in a murine model, with additional experiments in progress.

**Methods:** Using random and rational mutagenesis, libraries of CGL variants were generated, and superior clones were identified through a genetic selection where degradation of homocysteine to α-ketobutyrate confers a growth advantage to an engineered strain of E.coli. Using a high
methionine diet (2.5% w/w as opposed to the normal 0.6% w/w), hyperhomocysteinemia was induced in mice, creating a murine model to assess the efficacy of the developed enzymes.

**Results:** Administration of a single dose 50 mg/kg (IP) of our enzyme therapeutic in the high methionine diet mouse model significantly reduced tHcy from 380 ± 13 μM to 44.7 ± 4.8 μM within 24 hours. Dosing every 5 days for 30 days at 50 mg/kg (IP) resulted in a prolonged reduction in tHcy as shown in figure 1. Additionally the prolonged, frequent dosing did not result in weight loss or any other signs of toxicity.

**Discussion:** Administration of the enzyme successfully depleted tHcy in the diet-induced murine model without adverse effects. Experiments are ongoing to assess the efficacy of the enzyme in additional mouse models to further prove the efficacy of this treatment.

**Conflict of Interest:** George Georgiou and Everett Stone are inventors on intellectual property related to this work, and have an equity interest in Aeglea Biotherapeutics, a company pursuing the commercial development of this technology.

Enzyme was dosed every 5 days at 50 mg/kg (IP). Serum was collected prior to every injection and 24 hours post injection.

**SESSION 17: B vitamins, homocysteine and cardiovascular disease**

**062 J. David Spence** Lowering homocysteine levels to prevent stroke: unraveling the complexity of the evidence

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The early studies, VISP (2004) and NORVIT (2006), in older patients with worse renal function than later studies, showed no overall benefit with folate/B6/B12. Indeed, in NORVIT there was harm from cyanocobalamin. However, among VISP participants with good renal function (2005), there was a significant 34% reduction of events (stroke, myocardial infarction and vascular death) in participants with a baseline serum B12 above the median (denoting ability to absorb B12) receiving high-dose B vitamins, vs. those with a baseline B12 below the median receiving low-dose vitamins. In Hope-2 (2006), in younger patients with better renal function, folate/B6 and cyanocobalamin 1000mcg vs. placebo reduced the risk of stroke but not MI by 23% (2006). In the French SuFoIOM3 trial (2010), with younger patients with better renal function, folate/B6 and cyanocobalamin 20mcg daily reduced stroke by 43%. In the DIVINe study (2010), in patients with diabetic nephropathy, folate/B6/Cyanocobalamin 1000 mcg accelerated decline of renal function and doubled cardiovascular events. Spence and Stampfer suggested (2011) that harm from cyanide in cyanocobalamin among participants with renal failure may have obscured benefit in the early studies. Then in 2015 the Chinese CSPPT study in >20,000 adults taking 10mg enalapril (mean age 50y), folate alone, without B6 or B12, reduced first stroke by 21% over a median of 4.5 years, in a jurisdiction with no folate fortification. Among participants with LDL-C > 2mmol/L, stroke reduction was 31%. importantly, CSPPT showed benefit of folate alone in participants with poor renal function.
There can be no further doubt that B vitamins to reduce homocysteine reduce the risk of stroke. Metabolic B12 deficiency is common in older Western populations. Cyanocobalamin appears to be harmful in renal impairment; this include the elderly. Studies are therefore required using methylcobalamin.

O63 Ottar Nygård Plasma Homocysteine, B vitamin treatment and cardiovascular disease: still relevant to study?

O64 Joshua W. Miller Studies on the Influence of Folic Acid, Riboflavin, and the Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphism on Nitric Oxide Production and Blood Pressure

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Introduction: The MTHFR C677T polymorphism is associated with blood pressure (BP) and risk of hypertension. Riboflavin supplements reduce BP in individuals homozygous for the variant form of MTHFR (677TT).

Aims: 1) To investigate the influence of folic acid and riboflavin on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in murine macrophage RAW cells. 2) To determine the effect of the 677TT variant on BP in response to oral consumption of a salt solution in humans.

Methods: 1) RAW cells were grown in media containing 0.4mg/L riboflavin and 4.0mg/L folic acid (control), 0.04mg/L riboflavin (lowB2), or 0.4mg/L folic acid (lowFA) for 48 hours, and then exposed to LPS for 24 hours. NO production was measured by chemiluminescence assay and inducible NO synthase (iNOS) expression by quantitative PCR. 2) Twenty normotensive individuals [18-35y; 677TT (n=9), 677CC: (n=11)] were tested in a repeated measures design. On 6 separate days, BP was measured in sitting participants every 10 minutes for 60 minutes post ingestion of 475mL NaCl solution (157mM) or water. Plasma riboflavin was determined by HPLC.

Results: 1) After LPS exposure, NO production in the lowB2 and lowFA cells was 30-35% and 35-40% of control cells, respectively (p≤0.02). Expression of iNOS after LPS increased in all conditions. 2) In all subjects there was a 3.1±2.7 mmHg greater drop in systolic BP (SBP) after ingesting the NaCl solution compared with water (p=0.018). The decrease in SBP was greater for 677CC individuals compared with 677TT individuals: -8.18±3.62 vs. -6.59±2.15 mmHg (p=0.013). Plasma riboflavin was not different between the groups.

Discussion: LPS-induced NO production is reduced in riboflavin or folic acid-deficient RAW cells independent of iNOS expression. Reduction in SBP after NaCl ingestion is attenuated by the MTHFR 677TT variant, independent of riboflavin status. These results demonstrate the importance of the folate cycle in maintaining NOS function, and indicate a potential mechanism for the effects of MTHFR polymorphisms on BP.

Funding: NIH DC02995, Rutgers Dept. of Nutritional Sciences. The authors have no conflicts of interest to declare.

O65 Espen Ø. Bjørnestad Neopterin as an Effect Modifier of the Cardiovascular Risk & Robert Borsholm Associated with Total Homocysteine – a Two-Cohort Study of Patients with Coronary Heart Disease

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Introduction: Plasma total homocysteine (tHcy) is related to plasma neopterin, an indicator of interferon-γ (IFN-γ) mediated immune activation, and both biomarkers positively predict cardiovascular risk.

Aim: We sought to examine whether the association between tHcy and subsequent risk of acute myocardial infarction (AMI) was modified by circulating concentrations of neopterin and C-reactive protein (CRP) among coronary heart disease (CHD) patients.

Methods: By Cox modelling, we assessed the association between tHcy and risk of AMI in 4164 patients with suspected stable angina pectoris (SAP). Subgroup analyses were performed according to median levels of neopterin and CRP. An independent replication study was performed among 3749 patients with AMI at baseline.

Results: Median follow-up time was 7.3 and 8.3 years among patients with SAP and AMI, respectively. tHcy and neopterin were positively associated in both cohorts (rs=0.34 and rs=0.30 among SAP and AMI patients, respectively, both p<0.001). Plasma tHcy predicted AMI in both cohorts (hazard ratios [HR] [95% CI] per 1-SD increment of log-transformed tHcy 1.28 [1.20-1.37] and 1.12 [1.06-1.18] among SAP and AMI patients, respectively). However, the risk associations were confined to patients with plasma neopterin above the median (Pint<0.005 in both cohorts). Further, adding information on the interaction between tHcy and neopterin improved model discrimination and reclassification. tHcy and CRP were weakly related, and no effect modification was found by CRP.

Discussion: Among patients with suspected or verified CHD, tHcy predicted risk of AMI only in subjects with concomitantly elevated plasma neopterin. Our results motivate further research on the relationship between homocysteine metabolism, cellular immune activation, and atherothrombosis.

Conflict of interest: The authors declare that there is no conflict of interest.

SESSION 18: Future for the conference and research society
O66 Brian Fowler The International Conference on Homocysteine and One-Carbon Metabolism: Quo Vadis

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The aim of this presentation is to lay the scene for a debate and discussion on the future of this meeting, hopefully leading the way to a consensus on the way forward. We will try to define how the future should look for our scientific community, how to strengthen future conferences and whether we could establish a closer and continuous collaboration with external partners and sponsors.

As background the previous history of this conference, first held in Dromoland castle, Ireland in 1995, and lastly in Nancy in 2015, will be revisited. Considerations on previous moves in 1998 to form an official society (Henk Blom) and investigations on potential links to other long established societies (Anne Molloy) will be given.

Specific questions and proposals:
- Is there the need for a scientific society to be the formal organizer of the conferences, to be responsible for engaging in more permanent collaboration with industry and other scientific and patient societies, and possibly designating working groups to create clinical guidelines thereby establishing the society on the scientific world map?
- What is the future for the conferences on Homocysteine and One-Carbon Metabolism, and how should they be organized?
- What is the uniqueness of the conference?
- Where is the field heading, which areas have decreased in interest (e.g. homocysteine lowering and heart disease risk), which have increased (e.g. one-C metabolism in cancer?)
- Which other conferences deal with related areas (FASEB, Gordon, international pteridine etc)?
- How should such a society be organized including a democratically structured organising committee or?
- Funding, should permanent or continuous collaborations be aimed for, which partners and organizations? (Industry, other societies).

Thus in summary, we wish to provide a platform at the conference for creating a more sustainable and solid organization around our community and the conference.

**O67 Martyn Hooper The Pernicious Anaemia Society**

Martyn Hooper.
Founder & Chairman of The Pernicious Anaemia Society.

When it was formed in 2004, the Pernicious Anaemia Society’s original aim was simply to provide newly diagnosed patients with a Plain English explanation of their diagnosis. However, it soon became obvious that there were serious problems with the time taken for patients to be diagnosed and receive treatment based on the patient’s individual need – 33% of our members waited over 5 years for a diagnosis; 44% were initially misdiagnosed and 64% of our members are unhappy with their treatment.

We provide information, help and support for patients and their families and friends on a daily basis via a telephone helpline, a wide variety of downloadable leaflets (including advice on employment and education issues), and a fully moderated online forum. We also act as Expert Witnesses and Advocates in tribunals & hearings involving our members.

We Raise Awareness of the problems with the diagnosis and treatment of Pernicious Anaemia by Briefing Elected Representatives at all levels, Lobbying Government Departments (we were responsible for initiating the new Guidelines on Cobalamin and Folate from the British Committee for Standards in Haematology), Giving Presentations to Healthcare professionals (including Psychiatric Health workers, General Nurses, Dental Hygienists and Podiatrists), Organising Conferences and giving Presentations at other symposiums.

We are uniquely placed to work with researchers and can provide data gathered from surveys of our members, allow access to our database and recruit participants to take part in research programmes and focus groups.

We are a registered charity and have grown into a respected patient support and advocacy group with over 8,500 members from all over the world. We now have active local support groups not only in the UK but also in the United States. Our membership grows by an average of 3.2 new members every day.
Poster presentations
SESSION 10: Poster session 1

P01 Angela T. S. Wyse Methionine administration in pregnant rats causes memory deficit in the offspring and alters cell ultrastructure in cerebral tissue
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Introduction: Recent studies suggest that gestational hypermethioninemia may impair brain development during prenatal life.

Aim: In the present study we evaluated the effect of gestational hypermethioninemia on locomotor activity, anxiety, memory, and motivation for exploration of rat offspring using the following behavior tests: open field, inhibitory avoidance, and object recognition. Cell ultrastructure was analyzed in cerebral tissue.

Methods: Wistar female rats received methionine (2.68 μmol/g body weight) by daily subcutaneous injections during gestation. Control animals received saline. Cerebral tissue from 21 and 30 days-of-age pups were analyzed by electron microscopy. Another group was left to recover until the 30th day of life to perform behavior tests.

Results: Results from the open field task showed that pups exposed to methionine during intrauterine development spent more time in the center of the arena. Regarding to inhibitory avoidance task, the decrease in the step-down latency at 1 and 24 h after the training demonstrated that maternal hypermethioninemia impaired short-term and long-term memories of rat offspring. In the object recognition memory task, methionine administration during pregnancy significantly reduced the total exploration time of rat offspring. The test session also showed that the methionine reduced the recognition index. Electron microscopy revealed morphological alterations in the cerebral tissue at 21 and 30 days of age.

Conclusion: Our findings suggest that the cell ultrastructure changes caused by maternal hypermethioninemia may be, at least in part, associated to the memory deficit of rat offspring.

Conflicts of interest: The authors declare that they have no conflict of interest.
Supported by CNPq and FAPERGS, RS, Brazil.

P02 Olga Utyro Telomere Length Is Not Affected by the Cystathionine-β Synthase (Cbs) Genotype, Age, and Life Span In Mice
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Introduction: Biological aging is a result of systematic telomere length shortening (TLS) during chromosome replication. In humans, senescence is associated with age, cardiovascular disease (Fitzpatrick A et al., Am J Epidemiol 2007;165:14-21) and homocysteine (Richards JB et al., Atherosclerosis 2008;200:271-277; Zhang D et al., Atherosclerosis 2013;231:173-179). In mice severe hyperhomocysteinemia in Tg-I278T Cbs/- reduces life span (Gupta S et al., FASEB J 2009; 23:883-893) but it is not known whether TLS is involved.

Aim: To examine a hypothesis that shorter life span and hyperhomocysteinemia are associated with TLS in the cystationine-β synthase-deficient (Cbs/-) mice.
Methods: DNA was isolated from the blood (36-408-days old males and females, n=79), liver, and brain (107-114-days old females, n=6) of Tg-I278T Cbs-/- and Tg-I278T Cbs+/+ sibling controls by the standard phenol method, and was RNase A-treated. We used a qPCR assay, which measures telomere (T) signals and a single-copy gene (albumin) signals (S) in DNA samples to yield telomere length ratios (T/S) that are proportional to telomere length (Cawthon R, Nucleic Acids Res 2009;37(3):e21).

Results: We found that telomere length (T/S) in leukocytes (Figure), brain, and liver was not affected by the Cbs-/- genotype. Telomerase Tert mRNA was reduced 5-fold in the Cbs-/- (n=5) vs. Cbs+/+ (n=7) livers (p=0.004), but not in brains. We also found that leukocyte telomere length was not affected by age (Figure) or sex in Cbs-/- mice or Cbs+/+ sibling controls.

Discussion: Our findings show that telomere length shortening is unlikely to contribute to the reduced life-span in Cbs-/- mice. Thus, in contrast to humans, hyperhomocysteinemia does not lead to TLS in mice, regardless of reduced telomerase expression in the Cbs-/- liver. Premature death of Cbs-/- mice is not caused by telomere shortening but, most likely, by an independent, organ-specific pathology.


P03 Elin Strand Tryptophan and metabolites of the kynurenine pathway are associated with serum fatty acids in patients with stable angina pectoris

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Introduction: Metabolites of the kynurenine pathway are synthesised from the indispensable amino acid tryptophan. Dysregulation of the tryptophan metabolism is associated with a wide range of chronic lifestyle diseases, including cardiovascular disease. Inflammatory conditions increase the expression of indoleamine-pyrrole-2,3-dioxygenase (IDO), which catalyses the rate-limiting step in the tryptophan catabolism. Furthermore, both dietary and endogenic fatty acids (FAs) are involved in central metabolic processes, including the production of eicosanoids important for inflammatory and immune responses.

Aim: To explore the associations of plasma tryptophan and kynurenines with serum FAs among 1219 patients with stable angina pectoris.

Methods: Clinical and biochemical information were obtained from patients (73% males, median age 62) who underwent coronary angiography in 2000-2004. Tryptophan and kynurenine metabolites in plasma were measured by LC-MS/MS, while serum FAs were analysed by GC-MS/MS. Correlation coefficients were assessed using partial spearman rank correlation adjusted for age and gender.

Results: In the total cohort, plasma tryptophan and kynurenines were positively associated with the concentrations of serum saturated FAs (SFAs) and monounsaturated FAs (MUFAs), with the strongest observed association for hydroxyanthranilic acid (HAA). Weaker, yet significant, associations were observed with n-3- and n-6-polyunsaturated FAs (PUFAs).

Discussion: In this study, we demonstrate that plasma tryptophan and metabolites of the kynurenine pathway are correlated with serum FAs, with HAA having a particular strong association with SFAs and MUFAs. As both kynurenines and FAs are related to inflammation, our findings should
motivate future studies on their association, as well as potential interactions in relation to the risk of lifestyle diseases.

**Conflicts of interest:** The authors declare no conflict of interest.

**P04**  Ben D. Warner  **The effect of thiopurine toxicity on homocysteine (Hcys) metabolism**


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**Introduction:** S-adenosylmethionine (SAM) is a co-factor for the methylation of mercaptopurine (MP) to methylmercaptopurine (MMP) by thiopurine methyltransferase (TPMT). Low SAM and high Hcys have been associated with liver disease suggesting that alterations in concentrations of these compounds through thiopurines may be a cause of hepatotoxicity.1 Hepatotoxicity affects 5% of Inflammatory bowel disease (IBD) patients taking thiopurines and is overcome by switching to a lower dose of thiopurine and adding allopurinol (LDTA). We investigated the markers of Hcys metabolism in such patients.

**Method:** 18 IBD patients on thiopurines had plasma SAM, SAH, Hcys, Methionine (Met) and 5-methyltetrahydrofolate (5-MTHF) measured. All were wild type for TPMT. 6 patients were hypermethylating with hepatotoxicity (ALT > 56 IU/L) at the time compounds were measured. 3 of these patients had measurements repeated at least 6 weeks after LDTA. The results were compared with 5 healthy controls. Plasma was separated from whole blood within 1 hour of collection on ice and frozen at -72°C. Compounds were measured using isotope dilution liquid chromatography mass spectrometry-mass spectrometry (LCMS/MS). 5-MTHF was measured using HPLC. Paired and independent T-tests determined differences between analytes.

**Results:** There were no age or weight difference between the groups. SAM was higher in patients on thiopurines compared to controls and SAM increased after LDTA was added (P < 0.05). Met, Hcys and MTHF did not alter between the groups. Concentration of SAM in controls was consistent with that shown in other studies on healthy patients.

**Table 1:** The mean (SD) of the analytes measured and patient characteristics within each group.

**Discussion:** These results suggest that the Hcys cycle may be disrupted by thiopurines and by LDTA independent of 5-MTHF concentrations. Since altered SAM have been attributable to liver disease, disruption of this cycle by thiopurines is a potential mechanism by which hepatotoxicity could be occurring.

No conflicts of interest

**References:**
2. Struys EA, Jansen EE, de Meer K, Jakobs C. Determination of S-adenosylmethionin

**P05**  Izabela Bielińska  **Cystathionine β-synthase deficiency induces changes in the mouse brain proteome associated with neurodegenerative disease**

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Introduction: Homocysteine (Hcy), an intermediate in one-carbon metabolism, is converted by cystathionine β-synthase (CBS) to cystathionine. Human CBS deficiency, a recessive inborn error in Hcy metabolism, causes severe hyperhomocysteinemia and leads to cardiovascular and neurodegenerative diseases. However, mechanisms underlying these pathologies are not fully understood.

Aim: Our aim was to test a hypothesis that CBS deficiency induces changes in gene expression that impair brain homeostasis and cause neurodegeneration.

Methods: We used severely hyperhomocysteinemic Tg-I278T Cbs-/-(n=16) and their littermate controls (n=16) (Gupta S et al., FASEB J 2009;23:883-893). Mouse brain proteomes were analyzed using label-free relative quantitative mass spectrometry. Proteins with a minimum of two identified peptides and p values <0.05 were considered as differentiating. Bioinformatic analyses were carried out using PANTHER and DAVID resources.

Results: We identified 85 mouse brain proteins whose expression was significantly altered as a result of the Cbs gene inactivation. Of these, 26 were up-regulated (e.g., ApoE, Glo1, Gstm1, Fth1, Ppa1) and 59 down-regulated (e.g., Dynll2, Eef1b, Eef1d, Eef1g, Acat1, Pcm1, Ndufa4, Cox6b1) in Cbs-/− mice. Some of these proteins affected by the Cbs-/− genotype (Park7, Ube2l3, Ndufa4, Ndufs6) are involved in Parkinson’s disease in humans. The GO analysis revealed that the affected proteins participate in protein folding and localization (Calr, Hspp9, Pfn1), generation of precursor metabolites and energy (Pfkp, Pfkm, Cs), and oxidation/reduction (Fth1, Aldh6a1). The KEGG pathways overrepresented in the Cbs-/− mouse brain are pyruvate (14.9-fold) and propanoate (16.3-fold) metabolism, antigen processing (6.7-fold), oxidative phosphorylation (4.7-fold), Parkinson’s disease (4.6-fold), fatty acid (8.1-fold) and amino acid (6.9-10.6-fold) metabolism (Figure).

Conclusions: These findings suggest that brain Cbs interacts with diverse cellular processes (Figure) essential for normal brain homeostasis and that deregulation of these interactions by the Cbs-/− genotype underlies the involvement of hyperhomocysteinemia in neurodegeneration.

Conflicts of interest: The authors declare no conflict of interest.


P06 Marta Sikora Label-free proteomic analysis of plasma of cystathionine β-synthase deficient mice and humans reveals changes in blood coagulation, hemostasis and antioxidant activity

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Introduction: Inborn errors in human Hcy metabolism due to mutations in the cystathionine β-synthase (CBS) gene induce severe hyperhomocysteinemia (HHcy), which causes pathologies in multiple organs, including the cardiovascular system, and leads to premature death due to thrombotic complications. However, mechanisms underlying the propensity to thrombosis in HHcy are not fully understood.

Aim: To examine changes in blood coagulation/homeostasis proteins as a possible pro-thrombotic mechanism in CBS deficiency.
**Methods:** We used label-free mass spectrometry to quantify changes in plasma proteomes of CBS-deficient patients (n=10) relative to unaffected their siblings/parents controls (n=14). We also analyzed plasma proteomes of Tg-I278T Cbs-/ mice (n=12) and their Tg-I278T Cbs+/- littermate controls (n=12). Proteins considered as differentiating were those with at least two identified peptides and p values <0.05. Bioinformatic analyses were carried out with PANTHER, DAVID, and Ingenuity Pathway Analysis (IPA) resources. We also examined by mass spectrometry fibrinogen N-homocysteinylation in CBS-deficiency.

**Results:** In humans, we identified 50 plasma proteins whose expression was significantly altered by the inactivation of the CBS gene. In mice, 75 plasma proteins affected by Cbs genotype were identified. In humans, top molecular pathway affected by CBS gene inactivation was blood coagulation and hemostasis involving coagulation factor XIII, alpha-1-antitrypsin, antithrombin III, kininogen, and heparin cofactor. Inactivation of the Cbs-/- gene in mice also affected the blood coagulation and homeostasis pathway involving coagulation factor X, heparin cofactor, antithrombin III, angiotensinogen, alpha-1-antitrypsin. In addition, an antioxidant protein, glutathione peroxidase 3, was upregulated both in plasma of CBS-deficient mice and humans. We also found that in fibrinogen isolated from CBS-deficient patients three lysine residues (α-Lys562, β-Lys344, γ-Lys385) were N-homocysteinylated. Of these residues, α-Lys562, is located in a region involved in tPA and plasminogen binding.

**Conclusions:** These findings explain at least in part the propensity to thrombosis associated with CBS-deficiency in humans.


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**P06 Vegard Lysne Exploring Dimethylglycine, Methylmalonic Acid and Nicotinamide as Predictors of Acute Myocardial Infarction and Mortality**

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**Introduction:** Elevated plasma dimethylglycine (DMG) has been shown to predict incident acute myocardial infarction (AMI) and mortality in patients with established cardiovascular disease. Increased activation of the nuclear receptor PPARα has been suggested as an underlying mechanism. In rats we observe a substantial increase in DMG after PPARα activation, with concomitant increases in methylmalonic acid (MMA) and nicotinamide (NAM, vitamin B3) levels.

**Aim:** To evaluate DMG, MMA and NAM as predictors of AMI and mortality, as well as exploring potential interactions between the biomarkers.

**Methods:** Patients undergoing coronary angiography for stable angina pectoris (n = 4063, 72 % male, median age 62, median follow-up 10.3y) were followed for incident AMI and all-cause mortality. Associations between baseline concentrations of DMG, MMA and NAM and endpoints were assessed with cox regression, adjusted for age, gender, current smoking, diabetes, hypertension, extent of coronary artery disease and plasma creatinine. Risk estimates are presented as Hazard Ratios (95% CI) per SD increase of the log-transformed predictor metabolite.

**Results:** During follow-up, increased DMG (1.14 [1.06-1.23]) was associated with increased risk of AMI, while MMA (1.06 [0.98-1.14]) and NAM (1.00 [0.92-1.08]) were not. For mortality, elevated plasma DMG (1.09 [1.02-1.16]) and MMA (1.15 [1.09-1.22]) were associated with increased risk, while plasma NAM was not (1.02 [0.95-1.09]). No statistically significant interactions were observed between the metabolites in relation to AMI risk or all-cause mortality in the multivariate models.

**Discussion:** In this prospective cohort study, both elevated DMG and MMA were associated with increased risk of mortality. Elevated DMG was also associated with increased risk of AMI. NAM was not associated with risk of either endpoint. These data may suggest that excess PPARα activation alone cannot explain the observed risk associations.

**Conflicts of interest:** None
Gene-specific DNA methylation in the offspring in response to folic acid supplementation during the second and third trimesters of pregnancy: evidence from a randomised controlled trial.

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Introduction: The role of folate in preventing neural tube defects in early pregnancy is well established but emerging evidence by ourselves and others suggests that maternal folate status may contribute to cognitive development in childhood. In recent years, it has been suggested that the effects of maternal folate on offspring cognition may be explained through epigenetic mechanisms. Previous studies using a candidate-gene approach to link folate status during pregnancy with offspring DNA methylation have reported significant associations for certain genes.

Aim: The current study aimed to investigate the effect of maternal folic acid supplementation on cord blood DNA methylation of genes related to brain function and growth. In addition, we considered the relationship of DNA methylation with cognitive performance of the offspring.

Methods: We analysed cord blood samples (n=86) from our previous trial of Folic Acid Supplementation in the Second and Third Trimesters (FASSTT) for DNA methylation levels of LINE-1, RBM46, PEG3, IGF2, GRB10, BDNF, GRIN3B, OPCML and APC2. We then linked the DNA methylation data with cognitive performance of the children at age 7 years, as previously assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI).

Results: The results showed significantly lower DNA methylation for LINE-1, IGF2 (Figure) and BDNF in the offspring of mothers who received folic acid treatment compared to placebo. In multiple linear regression analysis (data not shown), factors found to significantly influence offspring DNA methylation of specific genes (apart from maternal folic acid treatment) were cord vitamin B12 concentration (IGF2) and caesarean section (LINE-1, RBM46 and BDNF). Furthermore, DNA methylation of cord blood was found to be significantly related to cognitive performance of the child for LINE-1 (Full Scale IQ) and IGF2 (Verbal IQ).

Discussion: These results indicate that folic acid supplementation (400μg/d) in trimesters 2 and 3 of pregnancy exerts significant effects on DNA methylation of specific genes in the offspring and thus offers a potential biological mechanism linking maternal folate with childhood cognition.

Funding: This study was conducted as part of the EpiFASSTT project supported by joint funding from the Biotechnology and Biological Sciences Research Council (BBSRC) and the Economic and Social Research Council (ESRC). For more details, please visit: www.bristol.ac.uk/essn. No conflicts of interest to declare.
**Introduction:** One-carbon metabolism and the related B-vitamins may be important for maintaining cognitive health in ageing but few studies have investigated the biomarker status of all the relevant B-vitamins.

**Aim:** The aim of this study was to examine the role of baseline status of folate and the metabolically related B-vitamins (vitamin B12, vitamin B6 and riboflavin) as predictors of cognitive decline over a subsequent five-year follow-up period.

**Methods:** From the total sample recruited (n=5186) to the Trinity, Ulster, Department of Agriculture (TUDA) Ageing Cohort study (2008-2012), from across the Island of Ireland, a sample of 2093 participants in Northern Ireland were potentially available, and from which a sub-sample (n=587; fulfilling the inclusion criteria) were reinvestigated 5 years after the initial TUDA study. The rate of cognitive decline was evaluated by re-assessment of cognition using the original battery of tests including the Mini-Mental State Examination (MMSE), the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and the Frontal Assessment Battery (FAB).

**Results:** At baseline, elevated plasma homocysteine and lower B-vitamin status were associated with cognitive dysfunction (MMSE score <25) after adjustment for age, education and sex. When the rate of cognitive decline at the 5 year follow up was examined, lower biomarker status at baseline (i.e. below median value) of vitamin B6 and riboflavin, but not folate or vitamin B12, were significant predictors (after adjustment for covariates), of accelerated cognitive decline, as measured by MMSE and RBANS but not FAB (Table).

<table>
<thead>
<tr>
<th>B-vitamin biomarker status</th>
<th>Median value at cut point</th>
<th>MMSE Accelerated cognitive decline</th>
<th>RBANS Accelerated cognitive decline</th>
<th>FAB Accelerated cognitive decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Homocysteine (μmol/L)</td>
<td>Reference &lt;13.0 vs 13.0-27.5</td>
<td>0.93 0.63-1.38</td>
<td>1.00 0.67-1.51</td>
<td>0.97 0.65-1.45</td>
</tr>
<tr>
<td>RBC Folate (μmol/L)</td>
<td>Reference &gt;868 vs 185-865</td>
<td>1.02 0.67-1.33</td>
<td>1.21 0.80-1.83</td>
<td>0.88 0.58-1.34</td>
</tr>
<tr>
<td>Serum Total B12 (pmol/L)</td>
<td>Reference &gt;252 vs 58-251</td>
<td>1.13 0.76-1.67</td>
<td>0.83 0.56-1.25</td>
<td>1.14 0.76-1.69</td>
</tr>
<tr>
<td>Plasma PLP (B6) (μmol/L)</td>
<td>Reference &gt;61.3 vs 11.6-61.3</td>
<td>1.82 1.08-2.43</td>
<td>1.75 1.16-2.65</td>
<td>0.98 0.65-1.47</td>
</tr>
<tr>
<td>EGRac (B2)</td>
<td>Reference &lt;1.30 vs 1.30-2.03</td>
<td>1.00 0.68-1.48</td>
<td>1.63 1.09-2.45</td>
<td>1.18 0.79-1.76</td>
</tr>
</tbody>
</table>

Binary logistic regression was performed with adjustment for age, education, sex, depression (CES-D score) and baseline cognitive score. 1Accelerated decline: Rate of cognitive decline in the highest quartile (25%) for each cognitive test. 2EGRac is a functional indicator of riboflavin status; a higher EGRac ratio indicates lower riboflavin status.

**Discussion:** These findings suggest that vitamin B6 and riboflavin have important roles in maintaining cognitive function in older people with generally good status of folate and vitamin B12. Optimisation of B-vitamin status, through regular consumption of fortified foods or supplements, may offer a means of protecting cognitive health in ageing.

**Funding:** Supported by governmental funding from the Irish Department of Agriculture, Food & the Marine and Health Research Board (under its FIRM initiative) and from the Northern Ireland Department for Employment and Learning (under its Strengthening the All-Ireland research base initiative). No conflicts of interest to declare.

**P10 Emma O’Sullivan** Impact of the MTHFR 677C→T polymorphism and its interaction with riboflavin on blood pressure in pregnant and non-pregnant women

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Introduction: Hypertension affects 10-15% of pregnancies and can lead to more serious hypertensive disorders with unfavourable pregnancy outcomes. Meta-analyses have reported that the common 677C→T polymorphism in the MTHFR gene is associated with an increased risk of hypertension in pregnancy. Previous trials from this centre in non-pregnant hypertensive patients have shown a blood pressure lowering effect in response to riboflavin supplementation that is specific to individuals with the MTHFR 677TT genotype. To date this relationship has not been examined in pregnant women.

Aim: The aim of this study was to investigate blood pressure in relation to the MTHFR 677C→T polymorphism and its interaction with riboflavin status in both pregnant and non-pregnant women.

Methods: Data for this study were derived from two pre-existing cohorts, namely the Irish National Adult Nutrition Survey (NANS) and the Folic Acid Supplementation Trial in the Second and Third Trimester (FASSTT) datasets.

<table>
<thead>
<tr>
<th></th>
<th>CC (n=154)</th>
<th>CT (n=150)</th>
<th>TT (n=43)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.9 (9.6)</td>
<td>34.4 (9.7)</td>
<td>34.3 (10.0)</td>
<td>0.826</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.4 (4.5)</td>
<td>25.6 (4.9)</td>
<td>25.9 (3.7)</td>
<td>0.765</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>110.5 (11.6)</td>
<td>113.4 (12.9)</td>
<td>117.2 (13.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>diastolic</td>
<td>73.3 (9.6)</td>
<td>76.0 (10.1)</td>
<td>78.3 (11.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Lower Riboflavin Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>109.5 (12.2)</td>
<td>113.5 (12.2)</td>
<td>119.3 (15.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>diastolic</td>
<td>72.8 (9.5)</td>
<td>76.3 (10.5)</td>
<td>79.6 (12.3)</td>
<td>0.025</td>
</tr>
<tr>
<td>Higher Riboflavin Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>111.5 (11.0)</td>
<td>113.2 (13.6)</td>
<td>114.6 (10.8)</td>
<td>0.326</td>
</tr>
<tr>
<td>diastolic</td>
<td>73.7 (9.7)</td>
<td>75.8 (9.8)</td>
<td>76.4 (9.7)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD)

*Statistical significance for comparison between genotype groups using 1-factor ANCOVA with adjustments for age and BMI. Values within a row with different letters indicate significant differences by a Bonferroni post hoc test. The chi-square test was used for categorical variables.

Results: In non-pregnant women (Table), those with the TT genotype had significantly higher systolic and diastolic blood pressure compared to women with the CC genotype. This effect was influenced by prevailing riboflavin status, such that differences among the 3 genotypes were greatest in those with lower riboflavin status. In contrast, genotype differences in blood pressure were not significant in participants with higher riboflavin status. Pregnant women (data not shown) with the TT genotype combined with a lower riboflavin status were found to have not only a significantly higher blood pressure, but also a greater increase in diastolic blood pressure from 14GW to 36GW compared to women with the CC/CT genotype (11.0 vs 4.2mmHg; P=0.013).

Discussion: These results suggest that the MTHFR 677TT genotype adversely influences blood pressure in women of reproductive age and during pregnancy. A higher riboflavin status appears to attenuate the effect of this genetic variant on blood pressure. A randomised controlled trial in pregnant women is necessary to confirm these findings.

Conflict of Interest: None to declare

P11 Jean-Louis Guéant Deciphering the methylome landscape of patients with cblC: Results from an epigenome wide association study using Infinium HumanMethylation450 BeadChip array
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France; 2Department of Human Genetics, McGill University and Research Institute McGill University Health Centre, Montreal, Quebec, Canada; 2INSERM, UMR_S937; ICAN Institute, Université Pierre et Marie Curie, Paris, France; 3INSERM, UMR_S1062, Nutrition Obesity and Risk of Thrombosis, Aix-Marseille University, Marseille, France.

Introduction: The genome-wide methylation landscape of patients presenting with cblC type methylmalonic academia with homocystinuria has never been reported.

Aim: We sought to decipher alterations of DNA methylome in cblC disease.

Methods: We typed whole-blood DNA from patients with cblC disease and relatives with heterozygous MMACHC genotype using the Infinium HumanMethylation450 BeadChip array (M450K). Methylation profiles were compared to a reference population using in silico data from 350 unrelated white patients of northern European origin from the MARTHA cohort. The comparison of CpG beta values was carried out using a t-test, and multiple testing corrections were performed using the Bonferroni adjustment. To assess population stratification according to whole methylome profile, numeric principal component analysis (PCA) was performed using normalized beta values of each CpG probe across the M450K. The eigenvalues of the top two principal components were used for the PCA plot.

Results: The patients presenting with cblC disease had a methylation landscape strikingly different from control subjects as seen on the PCA plot. The genome-wide methylation landscape of subjects with heterozygous MMACHC mutation did not differ from that of control subjects. A total of 1,145 CpG probes had a hypermethylated signature with an absolute difference of beta values > 0.7, corresponding to 452 genes. Using the PANTHER overrepresentation test tool (geneontology.org), this set of 452 genes was enriched for developmental process, ectoderm development, regulation of transcription from RNA polymerase II promoter, signal transduction, and cell communication. Hypomethylation < –0.7 was observed in 5,566 CpG probes located on 2,069 genes with enrichment for JNK cascade, anatomical structure morphogenesis, induction of apoptosis, developmental processes, mesoderm development, nervous system development, cellular component morphogenesis, cell adhesion and intracellular signal transduction.

Discussion: The methylome of cblC showed a dramatic predominance of hypomethylation signatures, and altered methylation profiles in genes involved in the development of ectoderm, mesoderm and nervous system. These preliminary results will deserve further investigation on the influence of the methylome on gene expression.

Conflicts of interest: The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

P12 Nuno Mendonça Elevated total homocysteine and plasma vitamin B12 concentrations are associated with all-cause and cardiovascular mortality in the very old: The Newcastle 85+ Study

Nuno Mendonça1,2,3, Carol Jagger2,4, Antoneta Granic5,7, Carmen Martin-Ruiz2, John C. Mathers2,3,7, Chris J. Seal1,3, Tom R. Hill1,3.

1School of Agriculture Food and Rural Development, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; 2Newcastle University Institute for Ageing, Newcastle University, Newcastle upon Tyne NE2 4AX, UK; 3Human Nutrition Research Centre, Newcastle University, Newcastle upon Tyne NE2 4HH, UK; 4Institute of Health and Society, Newcastle University, Newcastle upon Tyne NE2 5PL, UK; 5Institute of Neuroscience, Newcastle University, Newcastle upon Tyne NE2 4HH, UK; 7NIHR Newcastle Biomedical Research Centre, Newcastle University and Newcastle upon Tyne NHS Foundation Trust, Newcastle upon Tyne NE4 5PL, UK; 7Institute of Cellular Medicine, Newcastle upon Tyne NE2 4HH, UK.
**Introduction:** Folate and vitamin B12 are key to the correct functioning of one-carbon (1-C) metabolism. The current evidence on associations between 1-C metabolism biomarkers and mortality is inconclusive and generally based on younger populations.

**Aim:** This study aimed to determine the associations between biomarkers of 1-C metabolism and all-cause and cardiovascular (CVD) mortality in the very old.

**Methods:** The Newcastle 85+ Study is a prospective longitudinal study of participants aged 85 years at recruitment living in Northeast England. Baseline red blood cell folate (RBC folate), plasma vitamin B12 and total homocysteine (tHcy) concentrations were available for 752-766 participants. Associations between biomarkers of 1-C metabolism and all-cause and CVD mortality for up to 9 years were assessed by Cox proportional hazard models and confirmed by restricted cubic splines.

**Results:** Participants with higher tHcy concentrations had twice the risk of death from any cause than those with lower concentrations (e.g. Q4 vs. Q1, HR: 2.05, 95% CI: 1.51-2.77, p<0.001) after adjustment for sociodemographic, lifestyle and health variables. Women with elevated plasma vitamin B12 concentrations (>500 pmol/L) had increased risk of all-cause mortality (HR: 1.70, 95% CI: 1.13-2.56, p=0.011) compared with those with concentrations 148-500 pmol/L.

**Discussion:** Higher concentrations of tHcy and plasma vitamin B12 were associated with increased risk of all-cause and CVD mortality in the very old. This confirms findings for tHcy in younger populations but the adverse relationships between elevated plasma vitamin B12 concentrations and mortality in this population are novel and require further investigation.

**Conflicts of interest:** The authors report no conflict of interest.

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**P13 Ömer Özcan**

**Lower serum formate levels in patients with Parkinson’s disease**

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**Introduction:** Formate has been found to transfer one carbon units from serine amino acid in mitochondria to the cytoplasm. Therefore serum formate levels were accepted as a marker for the mitochondrial one carbon metabolism. Thus a sensitive and photometric serum formate measurement technique is required for the assessment of mitochondrial one carbon metabolism.

**Aim:** To optimize photometric serum formate determination method and by using this technique to measure blood formate levels which are the end product of mitochondrial one carbon metabolism in order to assess one carbon metabolism in patients with Parkinson’s disease.

**Methods:** In 60 Parkinson’s disease patients and 60 healthy individuals, folate, vitamin B12, erythrocyte ferritin, urinary MMA, serum ferritin, TIBC, serum iron, RBC folate, homocysteine, MTHFR C677T and MTHFR A1298C mutation tests and serum formate concentrations were measured. We optimized the sample preparation step and reagent compositions of photometric formate determination method.

**Results:** Our new optimized photometric formate measurement method was linear between 0-1mM formate ranges, reproducibility was between 13.9% and 20%, and recovery was between 84% and 92%. It has enough sensitivity for the low formate concentrations (detection limit; 0.05 mM). Blood formate levels of Parkinson’s patients were found significantly lower than in healthy individuals. (0.414 ± 0.314 mM, and 0.615 ± 0.267 mM, respectively, p = 0.000).

**Discussion:** The significantly lower serum formate levels in Parkinson’s disease may be an indicator of improper functioning of mitochondrial single carbon metabolism. In addition, by using our new optimized formate measuring method, the measurement of the concentration of the formate molecules in other diseases containing mitochondrial dysfunction, comparing their levels in the different stages of those diseases and also in other diseases having defective single carbon metabolism might give us important information about the functioning of mitochondrial one carbon metabolism.

**Conflict of Interest:** None
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Introduction: Worldwide, >200 million individuals are exposed to arsenic, an established carcinogen which has also been associated with cardiovascular disease. There is growing evidence that B vitamins enhance arsenic metabolism and reduce risk for arsenic-related health outcomes, including cancer and hypertension. However, little is known about the effects of B vitamins on intermediates of arsenic toxicity in the U.S., where B vitamin intakes tend to be high due to folic acid fortification and the widespread use of supplements.

Aim: To examine the impact of B vitamin intake on arsenic metabolism and urinary isoprostane F2-IsoP, an indicator of oxidative stress, in a subset of U.S. adults from the New Hampshire Health Study (median age 66).

Methods: Nutrient intakes were determined by food frequency questionnaire. Weighted Quantile Sum regression models, adjusted for age, smoking status, sex, BMI, education, calories, and batch (F2-IsoP), were used to examine the combined effect of thiamine, riboflavin, vitamin B6, vitamin B12, folate, and niacin intakes from food sources on methylated arsenic metabolite proportions in urine (%MMA, %DMA) and F2-IsoP (n=383). Using bootstrapping, we calculated weights for each nutrient to identify the most influential B vitamins. We also compared results including B vitamin intakes from supplements.

Results: Individuals with a higher %MMA, a risk factor for many arsenic-related health outcomes, had higher F2-IsoP measures (β: 0.11, P=0.03). Overall B vitamin intake from food sources was inversely associated with %MMA (β: -0.15, P=0.03) and F2-IsoP (β: -0.22, P<0.0001), but positively with %DMA (β: 0.02, P=0.02), which has been associated with reduced risks for many arsenic-related health outcomes. The 6 vitamins contributed relatively equally to these effects. Associations were somewhat attenuated when intakes from supplements were included.

Discussion: Higher dietary B vitamin intakes, particularly from food sources, appear to enhance arsenic metabolism and reduce oxidative stress among older U.S. adults.

Conflicts of Interest: The authors declare that they have no conflicts of interest.
Introduction: Homocysteine, an independent risk factor of osteoporosis and cardiovascular disorders, is an intermediate of the one-carbon pathway. The pathogenic mechanism might involve deregulation of DNA methylation. We investigated the association between mildly elevated plasma homocysteine and epigenome-wide DNA methylation in leukocytes.

Methods: DNA methylation was measured using Illumina 450k arrays in 2,035 individuals from 6 individual cohorts. Meta-analysis was performed to identify differentially methylated positions (DMPs) related to homocysteine. Differentially methylated regions (DMRs) were assessed using comb-p. Pathway analysis was performed using WebGestalt.

Results: Three DMPs were significantly associated with homocysteine. DMP cg21607669 located near the SLC27A1 gene at chromosome 19, had the lowest p-value (FDR=0.036). The 2 other DMPs were cg26382848 with nearest gene AJUBA located at chromosome 14 (FDR=0.037), and cg10701000 located at chromosome 10 (FDR=0.037). In addition, we identified 68 DMRs significantly associated with homocysteine. The most significant DMR was located at chromosome 6 near genes TNXB and ATF6B and spanned a region of 1.8 Kb containing 55 CpGs (Sidak P = 1.12E-21). Pathway analysis on the 114 genes annotated to the 68 DMRs showed 14 significant pathways related to folate biosynthesis, glycosaminoglycan biosynthesis, arachidonic acid metabolism and glycerophospholipid metabolism.

Discussion: We performed the first large-scale epigenome-wide analysis on plasma homocysteine and identified a modest number of differentially methylated positions in leukocytes. In addition, DMR analysis shows promising findings and the specific role of homocysteine in relation to these DMRs needs to be validated.

P16 Nafisa M. Jadavji MTHFR deficiency increases vulnerability and impairment in an aged mouse model of stroke

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Introduction: Stroke is a leading cause of disability and death world-wide. Increased levels of homocysteine have previously been associated with risk for stroke. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in folate and homocysteine metabolism. In all tissues, including in the brain, MTHFR catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methylTHF). The methyl group from 5-methylTHF is a substrate in the vitamin-B12-dependent methylation of homocysteine to form methionine by methionine synthase. A polymorphism in Mthfr (677C>T) has been identified in 5-15% of North American and European populations. Individuals with the TT genotype have elevated homocysteine concentrations and may have increased risk of stroke.

Aim: The aim of this study was to investigate the mechanisms through which MTHFR deficiency may increase risk of stroke.

Methods: Using primary glial cells derived from MTHFR-deficient mice we exposed cells to hypoxia, in vitro model of stroke. Twenty-four hours after damage, we measured cell viability. In vivo, using aged (~1.5-year-old) male Mthfr+/− and wildtype littermate controls, we damaged the sensorimotor cortex using a mouse model of stroke. Post-operatively, animals were tested on skilled motor function and brain tissue was processed to assess cell death, oxidative stress, and immune function.

Results: Primary glial cells from Mthfr−/− showed reduced cell viability through increased trypan blue
staining and MTT release. Increased immunofluorescence staining of active caspase-3 was also observed in Mthfr-/- cultures. In vivo, aged Mthfr+-/- mice showed impairment in skilled motor function after stroke. Mthfr-/- mice, despite of treatment, had significantly higher levels of plasma homocysteine compared to wildtype. In brain, at the damaged site, we observed increased expression of active caspase-3 and SOD2, a marker for anti-oxidant activity.

**Conclusion:** The data suggest that MTHFR deficiency increases vulnerability to stroke and leads to more severe impairment after stroke through increased apoptosis and oxidative stress.

**Conflict of Interest:** None

**P17 D. Sean Froese**  
Functional insights into severe MTHFR deficiency from characterization of 72 patient fibroblasts and 22 over-expressed mutations

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Introduction: 5,10-Methylenetetrahydrofolate reductase (MTHFR) catalyzes the NADPH-dependent reduction of 5,10-methylenetetrahydrofolate to 5-methylhydrofolate. The MTHFR protein comprises the N-terminal catalytic domain, responsible for enzymatic activity, and the C-terminal regulatory domain, which mediates allosteric inhibition by adenosylmethionine. Severe deficiency of MTHFR - associated with increased levels of homocysteine - represents an important disorder of folate metabolism.

Aim: To better understand how mutations in MTHFR cause disruption at the protein level, we characterized enzymatically: 1) fibroblasts from 72 different patients with severe MTHFR deficiency, and 2) 22 of the most common mutations and 2 common SNPs following over-expression in a MTHFR deficient fibroblast cell line.

Methods: Using the physiologically forward assay we determined the percent residual control activity as well as FAD-responsiveness, heat stability, Km for the substrates NADPH and methylenetetrahydrofolate and Ki for the allosteric inhibitor adenosylmethionine for endogenous MTHFR from patient cell lines. For the over-expressed mutant and SNP containing proteins we further performed Western blot analysis.

Results: Mutant MTHFR proteins displayed a spectrum of activities ranging from undetectable to 42% control in patient cell lines and up to 122% wild-type when over-expressed. There was good correlation of enzymatic parameters for individual mutations found in both studies. In general, proteins that had the lowest activity harboured either truncating mutations or homozygous missense mutations in the catalytic domain and had lower protein expression. By contrast, proteins that had high (>20% control) residual activity harboured at least one missense mutation in the regulatory domain and additionally had decreased affinity for NADPH (25 patients). Further, mutation islands found near the beginning and end of the regulatory domain were associated with altered Ki for adenosylmethionine (18 patients), suggesting discrete binding regions.

Discussion: This work provides valuable characterization of the molecular basis of MTHFR dysfunction, helps determine the significance of missense mutations and points to potential genotype-phenotype relationships.

Conflicts of interest: The authors declare no conflicts of interest.

Abstract Figure

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P18 Pieter H. Griffioen

Measurement of transcobalamin using a routine platform

Pieter H. Griffioen, Jan Lindemans and Sandra G. Heil*.
Erasmus MC, Department of Clinical Chemistry, workgroup Epigenetics.

Introduction: Vitamin B12 deficiency is mostly caused by insufficient gastro-intestinal absorption and in rare conditions by transcobalamin (TC) deficiency. Unsaturated transcobalamin (apoTC) can be measured by radiolabeled cobalamin. Recently, the Active B12 test has become available, which enables measurement of saturated transcobalamin (holoTC).

Aim: We hypothesize that this Active B12 test can be used to measure transcobalamin by additional in vitro saturation with cobalamin.

Methods: Serum was saturated in vitro by a 16 times dilution with cyanocobalamin and TC was selectively measured with the Abbott Active B12 test on the Architect. ApoTC was calculated by subtracting endogenous holoTC from TC after correction for dilution. Limit of quantification (LOQ) and linearity were determined with a pool serum dilution series. Precision was investigated according to CLSI EP15 protocol. Method comparison was performed against the binding assay using radiolabeled cobalamin. Reference values were determined in 100 healthy controls.
Results: LOQ of TC was determined at 88 pmol/L. The method showed linearity ranging 182-1077 pmol/L, \( R^2=0.995 \), lack of fit \( F=1.53 \). TC precision of low- and high-pool serum were; 5.2% and 4.3% respectively. Method comparison against radiolabeled cobalamin binding assay showed a proportional bias of 30% (\( Y= 126.18 + 0.70X \)). TC reference values were determined at 459-1340 pmol/L.

Conclusion: We describe a rapid, reliable and easy method to quantify TC, which can easily be implemented on routine platforms using commercial holoTC tests. In addition, apoTC can be easily assessed as well by subtracting endogenous holoTC concentration which can be measured in the same run on the same automated immunochemistry analyzer, securing the same calibration level for all three parameters. This testing strategy facilitates measurement of TC, apoTC and holoTC in clinical diagnosis but also in larger epidemiological studies.

Key words: Active B12, holo-Transcobalamin, apo-Transcobalamin, Vitamin B12.

Table 1: Total precision according to CLSI EP15 protocol for holoTC, apoTC and TC.

<table>
<thead>
<tr>
<th>Total Precision</th>
<th>holoTC</th>
<th>apoTC</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low pool-serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (pmol/L) CV(%)</td>
<td>31 ± 2</td>
<td>204 ± 11</td>
<td>236 ± 12</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>High pool-serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (pmol/L) CV(%)</td>
<td>118 ± 4</td>
<td>1075 ± 51</td>
<td>1193 ± 51</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>4.8</td>
<td>4.3</td>
</tr>
</tbody>
</table>

P19 Shyue-fang Cobalamin (Cbl) controls mRNA transport through its influence on Battaglia-Hsu methylation/phosphorylation of RNA binding proteins in brain
Battaglia-Hsu, SF*1, Ghemrawi, R, *1, Dreumont, N1, Coelho, D1, Gauchotte, G2, Sequeira, JM3, Ndiongue, M1, Houlgatte, R1, Helle D1, Alberto, JM1, Umoret, R1, Jung, M3, Quadros, EV2, Gueant JL1.
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Introduction: Cobalamin (Cbl)-related inherited metabolic disorders and nutritional deficiency occurring during pregnancy and ageing manifest severe neurological symptoms. The signaling pathway of cobalamin remains largely unidentified in brain.

Aim: We investigated whether the deregulation of subcellular distribution of mRNA binding protein (RBP) was related to the neurological manifestations associated with inherited Cbl disorders.

Methods: We examined in three models of impaired cellular metabolism of Cbl: TO/OT cells and Cd320 KO mouse and fibroblasts from patients with inborn errors of Cbl metabolism with defected Methionine Synthase activity.

Results: Impaired Cbl metabolism produced dramatic transcriptomic changes and impaired mRNA transport by blocking RBP nucleocytoplasmic shuttling in TO neuronal cells and in mouse brain cells with invalidated Cd320 receptor. These arose from the disruption of nuclear export of a key RBP involved in neuronal stress response through altering its interactions with CRM1/exportin within the nuclear pore complex. This export block was also present in fibroblasts from patients with inherited Cbl disorders. Our evidence suggested that decreased levels of S-adenosylmethionine and CARM1 expression, and increased level of PP2A expression are responsible for the block. They caused reduced methylation and phosphorylation of the RBP. The participation of CARM1 in the RBP methylation was evidenced by the siCARM experiments in OT cells where reduced R217 methylation of this RBP indeed blocked the RBP export. The PP2A inhibitor okadaic acid or knockdown of PP2A restored also the localization of the RBP in TO cells. The mislocalisation of this RBP led to decreased SIRT1 deacetylase expression and affected its RNA targets with functions in cell differentiation,
cellular stress, vesicular transport, and neurogenesis and amyloid protein pathways.

**Discussion:** These transcriptome-wide consequences are consistent with the role of Cbl in brain development, neuroplasticity and myelin formation and highlight SIRT1 as a therapeutic target in inherited disorders of Cbl metabolism.

**Conflict of interest:** None

**P20**

Laura Sposito: a tessera in the mosaic of molecular mechanisms of human cobalamin disorders.

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**Introduction:** Recently, cobalamin disorders have been associated with mutation in the X-linked *HCFC1* gene encoding the Host-Cell Factor (HCF-1)²⁻⁵. HCF-1 promotes cell proliferation and is an important component of a large number of nuclear complex involved in the regulation of gene expression. It associates with both DNA-binding proteins (E2F1/4, THAP11, ZNF143, …) and chromatin modifiers (Set1/Ash2, MLL/Ash2, Bap1, Sin3a, …) with either an activator or a repressor function. Thanks to this plasticity HCF-1 plays a role in both regulation of cell proliferation and metabolism. Recent findings suggest that HCF-1 regulates the expression of *MMACHC* causing the *cblX* phenotype.

**Aim:** As the precise role of HCF-1 in cobalamin metabolism is unclear, our project aims to understand how some but not all mutant *HCFC1* allele mutations cause the *cblX* defect and if they might cause also problem to cell proliferation.

**Methods:** Four *cblX*-associated and two non *cblX*-associated *HCFC1* missense mutations³ have been individually rebuilt via CRISPR-Cas9 homologous recombination into HEK-293 cells to create an isogenic cellular model. Gene expression patterns have been tested via RT-qPCR and their proliferative potential and HCF-1 effector protein interactions tested.

**Results:** The HCFC1 mutants display different temperature-sensitive proliferation phenotypes. All mutants grow normally at 33.5°C, whereas at 39.5°C two *cblX* mutants arrest cell proliferation whereas the other two *cblX* and the two non *cblX*-associated mutants grow as wt. The RT-qPCR mRNA analyses revealed that the *cblX* mutations cause a reduction in the expression of *MMACHC* consistent with the cobalamin metabolism disorder. Co-immunoprecipitation-immunoblot analyses have shown that only Y103H and T239M HCF-1 mutants decrease the interaction with Ash2 and Bap1.

**Discussion:** Our results confirm the correlation between *HCFC1* and cell proliferation as well as metabolism of cobalamin with a particular correlation with the residues involved in this processes, in fact our findings show that not all the human mutations affect cell proliferation, cobalamin metabolism and HCF-1 effector protein interactions in the same way indicating that HCF-1 plays varied roles in cell functions.

**Conflict of interest:** The authors have no conflicts of interest to disclose.


**P21**

Agata Sobczyńska- Malefora: The incidence of anaemia in 12-19 yr old female patients in South London, UK

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Introduction: Adolescent females are particularly at risk of developing anaemia because of inadequate/imbalanced nutrition associated with young age and compounded by menstrual blood losses. Iron deficiency is the most common cause of anaemia. Iron deficiency with and without anaemia is also linked to negative effects in cognitive development among adolescents. Although serum ferritin concentration is a sensitive indicator of iron deficiency, discrepancies exist with regards to lower cut-off limits in this cohort.

Aim: To evaluate the incidence of anaemia in 12-19 yr old female patients referred for ferritin testing. To evaluate a lower reference limit for ferritin, established using a modified Hoffmann’s approach1.

Methods: All ferritin results together with other markers of iron status, serum folate, vitamin B12, FBC and CRP, processed between Aug 2014-Jul 2015 on Architect i2000SR (Abbott Diagnostics) were obtained. Reference intervals (RIs) for ferritin were established using these data.

Results: In total, 1760 ferritin results were generated. The RI for ferritin was 7-75 μg/L. Results with a reference to anaemia are shown in Table 1. The median concentration (interquartile range) for ferritin, folate and B12 was 33 μg/L (18-67), 4.9 μg/L (3.5-6.7) and 413 ng/L (314-564) respectively.

Discussion: There was a high prevalence of anaemia (haemoglobin <120 g/L) and folate insufficiency in 12-19 yr old female patients. The RIs for ferritin calculated using a modified Hoffmann’s approach agreed with other published RIs values established using traditional methods. The estimated prevalence of iron deficiency using ferritin concentration alone varied from 3.7 to 31%, depending on the applied cut-offs. Serum iron and transferrin saturation in a small subset of patients with ferritin <7 μg/L confirmed iron deficiency in all cases, while, in a large proportion of patients with ferritin between 7-15 μg/L, these markers were also suggestive of iron deficiency. Confirmatory analysis with other markers of iron status should be applied for ferritin concentrations between 7-15 μg/L.

<table>
<thead>
<tr>
<th>N results</th>
<th>Test (&lt; =) cut-off suggestive of anaemia/acute phase</th>
<th>N (%)</th>
<th>cut-off suggestive of anaemia/acute phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>HB &lt;120 g/L</td>
<td>817 (42)</td>
<td>452 (43)</td>
</tr>
<tr>
<td>1951</td>
<td>RBC &lt;3.9x10^1²/L</td>
<td>464 (24)</td>
<td>377 (26)</td>
</tr>
<tr>
<td>1951</td>
<td>MCV &lt;80 µ/L</td>
<td>293 (15)</td>
<td>76 (7)</td>
</tr>
<tr>
<td>1951</td>
<td>MCHC &gt;27 µg/L</td>
<td>497 (25)</td>
<td>152 (15)</td>
</tr>
<tr>
<td>1951</td>
<td>RDW &gt;16</td>
<td>457 (22)</td>
<td>230 (22)</td>
</tr>
<tr>
<td>815</td>
<td>Serum folate &lt;3.1 µg/L</td>
<td>146 (18)</td>
<td>62 (17)</td>
</tr>
<tr>
<td>640</td>
<td>Serum B12 &lt;187 ng/L</td>
<td>34 (3.7)</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>1760</td>
<td>Serum ferritin &lt;22, &lt;15, &lt;7 µg/L</td>
<td>549 (31), 322 (18), 65 (3.7)</td>
<td>na</td>
</tr>
<tr>
<td>207</td>
<td>Fe &gt;11 µmol/L</td>
<td>74 (26)</td>
<td>30 (24)</td>
</tr>
<tr>
<td>195</td>
<td>TSAT &gt;18%</td>
<td>62 (32)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>195</td>
<td>TIBC &gt;77 µmol/L</td>
<td>181 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>356</td>
<td>CRP &gt;4 ng/mL</td>
<td>85 (25)</td>
<td>71 (31)</td>
</tr>
</tbody>
</table>

*30 μg/L, WHO suggested threshold for iron stores depletion in an acute phase or chronic disease; **15 μg/L, WHO suggested threshold for iron stores depletion; ^ 22 μg/L, cut-off currently used

Conflicts of interest: All authors declare no conflict of interest

References:
Gaucher disease (GD) is a lysosomal disorder caused by deficient activity of glucocerebrosidase due to mutations in the GBA1 gene. The clinical features associated to GD are hepatosplenomegaly, hematological disorders, bone changes and neurological involvement in disease types II and III. Patients with GD are usually treated with enzyme replacement therapy (ERT) or substrate reduction therapy. Some studies suggest a high incidence of low serum vitamin B12 (B12) in untreated Ashkenazi Jews with GD type I. Objective: To determine the prevalence of reduced levels of B12 and increased levels of total homocysteine (tHcy) in a cohort of Brazilian patients with GD.

Methods: A retrospective study was performed based on medical records of 41 patients with GD (Type I=37, Type II=1 and Type III=3; female=20; mean age: 36.3y) followed in a Reference Center for GD in Southern Brazil. No patient had Ashkenazi Jewish ascendency. B12 was measured on a yearly basis and tHcy every other year. Holo-transcobalamin (holo-TC) folate and methylmalonic acid measurements were not available.

Results: The mean number of B12 and tHcy measures available per each patient was, respectively, 6 and 2. All patients deny vegetarian/vegan habits. Nine (21.9%) patients had at some point only low B12 (<211 pg/mL, n=4; on ERT=3; untreated=1), high tHcy levels (>15 umol/L, n=3 all on ERT), or both (n=2; untreated). Regarding patients on ERT whose B12 levels improved afterwards (n=3), B12 supplementation was prescribed only for one. Data on the follow-up was available for 2 out of 3 untreated patients, which showed increase of B12 after ERT in both cases.

Conclusion: Our data suggest that low serum B12 levels are frequent in GD patients, even in regularly treated patients. The reasons for these findings are yet unknown. We hypothesize an impairment of the vitamin B12 transport through the lysosomal compartment due to the storage of glucosylceramide.
Case report: A 2-month-old male, first child born to a non-consanguineous couple, with unremarkable perinatal history, developed jaundice at 10 days of age and was admitted to our hospital to investigation. He gradually developed cholestasis, hepatosplenomegaly, ascites, sepsis, and thrombocytopenia. Clinical and laboratory tests confirmed the diagnosis of GD (chitotriosidase 5.0 nmol/h/mL, RV 8.8 – 132; B-glucosidase in leukocytes 0.65 nmol/h/mg protein, RV 10-45; GBA1 sequencing: L444P/RecNciI), and due to the GBA1 genotype and severity of the symptoms, GD type 2 was confirmed. At 60 days of life, the patient was found to present very high levels of ferritin (4,175 ng/mL, RV 30 – 400), abnormal liver function enzymes (AST 483 U/L, ALT 234 U/L), prolonged prothrombin time, and high levels of B12 1459 pg/mL (VR 211-946). Total serum homocysteine was 8.3 umol/L (VR 5-15) and methymalonic acid was 0.18 umol/L (VR 0.16-0.60). A second blood sample was analysed, and high levels of B12 and of holo-transcobalamin (holo-TC) were confirmed. The patient had never received vitamin supplementation, although he was fed with infant formula. A gene panel, including 16 genes involved in iron metabolism, identified 3 heterozygous non-pathogenic variants (p.Ser65Cys in HFE, p.Gln4His in FTL, and p.Val736Ala in TMPRSS6). After discharge, he stayed at home for 10 days and died at 76 days of life.

Discussion and conclusions: High level of ferritin is one of the hallmarks of GD, being considered a consequence of inflammation associated with the storage of glucocerebroside in macrophages. Although the high levels of B12 and holo-TC can be partially explained by the ingestion of infant formula, which contains relative high amounts of vitamin B12 and may potentially mask a “mild” B12 deficiency, this case suggests B12 metabolism may be impaired in GD, especially in the neuronopathic forms. Alternatively, increased B12 could be a marker of macrophage activation. The use of B12 and holo-TC as biomarkers of GD should be explored.

P24 Nahid Tamanna Effects of methionine restriction on hepatic homocysteine metabolism and H2S producing enzyme activities
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Introduction: ‘Methionine-restriction’ (MR), increases life span in diverse organisms. Despite low dietary sulfur amino acid content, rodents on MR develop ‘hyperhomocysteinemia’- a marker of cardiovascular and neurological diseases. Homocysteine can be remethylated to methionine or it can be metabolized to cysteine through the transsulfuration pathway. Cystathionine-β-synthase and cystathionine-γ-lyase (CGL) sequentially catalyze homocysteine to cysteine through transsulfuration. These enzymes, along with mercaptopyruvate sulfotransferase (MPST), are also the primary intracellular sources of the important messenger hydrogen sulphide (H2S), which has been associated with longevity.

Aim: Investigate the regulation of homocysteine metabolism in a rodent model of MR by measuring key metabolites related to homocysteine metabolism and enzyme activities associated with H2S production and the transsulfuration pathway.

Methods: Fischer-344 rats were fed with a control or MR diet for up to 8 weeks followed by tissue collection for biochemical analysis. Metabolites were measured by HPLC and enzyme activities were assessed via radio-isotopic assay or spectrophotometry. Production of H2S by CBS and CGL were determined by the zinc acetate trapping method.

Results: Homocysteine levels do not change in liver due to MR, however they increase in plasma. Additionally, reduced hepatic cysteine indicates a compromised transsulfuration pathway due to MR. S-Adenosylmethionine (SAM), an allosteric activator of CBS, is reduced in liver by MR leading to a decline in cellular methylation potential (SAM:S-adenosylhomocysteine) in liver. The activity of CBS decreases in the liver of MR animals while, unexpectedly, the activity of CGL increases. The activity of MPST also increases implicating a compensatory mechanism to maintain H2S production in response to declining CBS activity with MR.

Discussion: Reduced CBS activity and SAM supports the contention that transsulfuration flux is reduced by MR leading to hyperhomocystenemia. Increased CGL and MPST activity may be functioning as a compensatory mechanism for low CBS in order to maintain H2S production.

Conflicts of interest: The authors declare that there is no conflict of interest.
P25  Christine M. Pfeiffer  A simplified red blood cell folate calculation: comparison to the traditional calculation using data from NHANES 2007–2010
Mindy Zhang, Maya R. Sternberg, and Christine M. Pfeiffer.
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Introduction: Red blood cell folate (RBF) concentrations are an indicator of folate body stores and require 3 components for traditional calculation: whole blood folate (WBF), serum folate (SFOL), and hematocrit (Hct); [WBF–SFOL(1–Hct/100)]/(Hct/100). SFOL and/or Hct data are sometimes unavailable; hemoglobin (Hb) concentrations are generally available in nutrition surveys.
Aim: To assess whether a simplified calculation generated accurate RBF results.
Methods: Using SFOL, RBF, Hct, Hb, and mean cell Hb content (MCHC) data for participants ≥1 y in NHANES 2007–2010 (n ~ 17000), we conducted 5 simplified calculations: 1) ignore SFOL (WBF/Hct); 2) impute SFOL of 40 nmol/L (population median); 3) impute Hct of 40%; 4) estimate Hct from the ratio of Hb/MCHC; 5) impute SFOL and estimate Hct. We compared geometric mean RBF from these 5 approaches to traditionally calculated RBF results for the entire population and for women of reproductive age (WRA, 15–44 y).
Results: Compared to the traditional calculation, ignoring SFOL produced on average 5.6% (5.9% in WRA) higher RBF results; imputing SFOL produced 0% bias (-1.2% in WRA); imputing Hct produced 1.9% bias (-3.9% in WRA); estimating Hct produced 0% bias (0% in WRA); and the combination of imputing SFOL and estimating Hct produced 0% bias (-1.2% in WRA).
Conclusion: Ignoring SFOL in the calculation of RBF or using an imputed Hct value biased RBF results. Using the population median SFOL as an imputed value, or the estimated Hct, or a combination of the 2 parameters resulted in no or small biases when estimating central tendency in a population. However, the impact of a simplified approach of only measuring WBF and Hb needs to be further evaluated at the tails of the distribution, at accepted cutoff values, and in populations with moderate or low folate status.

P26  Zia Fazili  Use of a recombinant plant γ-glutamyl hydrolase enzyme eliminates the need for lengthy incubation of whole blood haemolysate samples prior to analysis of folate monoglutamates
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Introduction: Whole blood folate is polyglutamylated and requires deconjugation to monoglutamate folate for analysis by LC-MS/MS. Endogenous serum γ-glutamyl hydrolase (GGH) is traditionally used requiring a lengthy incubation time to achieve maximal recovery of folate monoglutamates. Recombinant GGH from Aribidopsis thaliana (AtGGH2) has shown promising results in analysis of food folates, however remains to be tested in whole blood samples.
Aim: To compare the efficiency of endogenous GGH with exogenous AtGGH in deconjugation of whole blood folate polyglutamates.
Methods: Whole blood folate samples (n=8) were diluted 1/11 in 1% ascorbic acid and incubated at 37°C for up to 4 h (conventional procedure) or diluted in 1% sodium ascorbate (pH 6.0) containing 10 μg/mL AtGGH2 and incubated at room temperature or 37°C for up to 90 min.
Results: Without incubation, total folate monoglutamate concentrations significantly differed between conventionally prepared samples (32.0±13.5 nmol/L) and AtGGH2 treated samples (53.1±21.9 nmol/L) (P<0.001). After 4 h a maximal concentration of 51.4±19.7 nmol/L was reached for conventionally prepared haemolysates that did not significantly differ to that of AtGGH2 treated samples at 0 h (P=0.170). However, there was evidence of some residual 5-methyltetrahydrofolate diglutamate (2.6±1.8 nmol/L) in conventionally prepared haemolysates even after 4 h, while 5-methyltetrahydrofolate diglutamate was not detected in AtGGH2 treated samples. Maximal concentrations of 5-methyltetrahydrofolate and 5,10-methenyltetrahydrofolate were achieved in AtGGH2 treated samples without incubation, however, some incubation may be required for full recovery of tetrahydrofolate and MeFox monoglutamates.
Discussion: Recombinant AtGGH2 resulted in faster deconjugation of folate polyglutamates to
monoglutamates than endogenous GGH, thereby reducing sample preparation time and potential degradation of folate. These findings require confirmation in a larger study and comparison with microbiologic assay results.

Conflicts of interest: None

SESSION 15: Poster session 2

P27 Joanna Perła-Kajan - N-homocysteinylation impairs collagen cross-linking in cystathionine β-synthase-deficient mice: a novel mechanism of connective tissue abnormalities

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Introduction: Cystathionine β-synthase (CBS) deficiency, a genetic disorder in homocysteine (Hcy) metabolism, elevates plasma Hcy-thiolactone (HTL) and leads to connective tissue abnormalities in humans affecting cardiovascular and skeletal systems. However, the underlying mechanism is not understood.

Aim: Because lysine residues participate in collagen cross-linking and HTL has the ability to N-homocysteinylate protein lysine residues, we tested a hypothesis that N-homocysteinylation prevents cross-linking of collagen.

Methods: We used a Tg-I278T-Cbs-/- mouse model, which replicates the connective tissue abnormalities observed in CBS-deficient patients. Plasma and urinary N-Hcy-protein, HTL, and total Hcy, collagen N-Hcy, S-Hcy, and pyridinoline cross-links were quantified by HPLC. Collagen was quantified by the hydroxyproline assay. Plasma deoxyxypirdinoline (DPD) crosslink, cross-linked carboxyterminal telopeptide of type I collagen (CTX I) and procollagen I C-terminal propeptide were quantified by ELISA. Collagen N-Hcy-Lys residues were identified by mass spectrometry. Lysyl oxidase (LOX) activity and mRNA expression were quantified by enzymatic assays and RT-qPCR, respectively.

Results: We found that N-Hcy-collagen was elevated in the heart, bone, and tail of Cbs-/- mice relative to littermate controls, while pyridinoline cross-links were significantly reduced. DPD and CTX I were also significantly reduced in Cbs-/- mice. LOX expression and activity were not affected by the Cbs-/- genotype. We also show that collagen carries S-Hcy bound to the thiol of N-Hcy (Figure). In vitro experiments show that Hcy-thiolactone modifies lysine residues in collagen type I α-1 chain. Residue K160, located in the non-helical N-telopeptide region and involved in pyridinoline cross-link formation, was also N-homocysteinylated in vivo.

Figure. Collagen N-homocysteinylation prevents cross-linking.

Discussion: Our findings show that N-homocysteinylation of collagen in Cbs-/- mice prevents its cross-linking (Figure). These findings explain at least in part connective tissue abnormalities observed in hyperhomocysteinemia. This work has been published (Perła-Kajan et al. FASEB J. 2016;30(11):3810-3821).

**P28 Sanjana Dayal**  
**Deficiency of Cystathionine beta Synthase Alters Blood Brain Barrier Integrity and Exacerbates Cerebral Ischemia Reperfusion Injury in Mice**

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**Introduction:** Hyperhomocysteinemia is a risk factor for stroke; however the mechanisms by which elevated homocysteine leads to stroke are poorly defined.

**Aim:** In a murine model of cystathionine beta synthase (CBS) deficiency, we investigated whether mild to severe elevation in plasma total homocysteine (tHcy) increases susceptibility to cerebral infarction and if this phenotype is associated with loss of blood brain barrier (BBB) integrity.

**Methods:** We studied male Cbs⁻/⁻ mice conditionally expressing a zinc-inducible mutated human CBS(I278T) transgene along with Cbs+/− and Cbs+/+ littermates at 10-14 weeks of age. The human transgene was allowed to express only until weaning to overcome the early mortality of Cbs⁻/⁻ mice. Experimental stroke was induced with middle cerebral artery occlusion for one hour followed by 24 hours of reperfusion. TTC stained coronal sections were used to quantify the infarcted area. BBB integrity was assessed prior to injury using a standard Evan’s blue (EB) infusion method. Leakage of EB was quantified in brain homogenates and normalized to brain weight.

**Result and Discussion:** Mild to severe increases in plasma tHcy were observed in Cbs+/− and Cbs⁻/− mice (6.1±0.3 and 309±18 μM, respectively) compared with Cbs+/+ littermates (3.1±0.6 μM, P<0.01). Both Cbs+/− and Cbs⁻/− mice exhibited significant increases in infarct size following ischemia-reperfusion injury as compared to Cbs+/+ mice (12.2±3% in Cbs+/+ mice vs. 35.1±7.7% in Cbs+/− mice, P<0.05). A significant increase in EB extravasation was observed in Cbs+/− and Cbs⁻/− mice (3.8±0.8 and 4.8±0.2 μg/g, respectively) compared to Cbs+/+ mice (1.7±0.4 μg/g, P<0.05). The similarity in extent of cerebral infarction observed in Cbs+/− and Cbs⁻/− mice suggests a threshold effect and that a mild increase in tHcy is enough to disrupt BBB integrity and produce a severe stroke phenotype in experimental murine models.

**Conflict of interest:** Authors disclose no conflict of interest

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**P29 Sandra G. Heil**  
**Reference values of vitamin B12, holoTC and MMA in 1,321 healthy women during third trimester pregnancy**

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**Introduction:** Reference values of vitamin B12 during pregnancy are not well described and are not used in clinical practice. Previous studies have shown that vitamin B12 levels are lower in pregnancy. In this study we aimed to determine references values of vitamin B12, holotranscobalamin (holoTC) and methylmalonic acid (MMA) in a large cohort of women with uncomplicated pregnancies.

**Methods:** Vitamin B12, holoTC and MMA levels were assessed in serum samples of 1,321 women in third trimester pregnancy (median 36±1 weeks) of the KOALA Birth Cohort Study, the Netherlands. Reference values were determined according to 2.5th and 97.5th percentiles using a non-parametric test (Analyse-it for Excel).

**Results:** Reference values of total vitamin B12 were 86-360 pmol/L, which are lower than current reference values in adults (148-637 pmol/L). HoloTC reference values were similar to those in non-pregnant adults; 95% reference interval 25-160 pmol/L compared to 21-117 pmol/L, respectively. The 95% reference interval of MMA was determined at 0.11-0.59 umol/L in pregnant women, which is slightly higher compared to current upper reference value of >0.45 μmol/L.

**Discussion:** Reference values of vitamin B12 in this population of pregnant woman were different from those in non-pregnant adults. We underline the need for additional reference values for vitamin B12 during pregnancy. HoloTC levels were not different in pregnant woman compared to normal population, suggesting that holoTC may be a better marker when screening for vitamin B12 deficiency in third trimester pregnancy.
The role of DNA hypomethylation in Sadenosylhomocysteine-induced endothelial activation

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Introduction: Hyperhomocysteinemia is an independent risk factor for atherosclerosis and cardiovascular disease by mechanisms still incompletely defined. S-Adenosylhomocysteine (SAH) is a potent methylation inhibitor that accumulates in the setting of hyperhomocysteinemia and may contribute to its vascular toxicity.

Aim: To investigate the effects of SAH in endothelial activation, and assess whether DNA hypomethylation contributes to these effects.

Methods: Intracellular SAH accumulation was induced in human endothelial cells (HUVEC) using a pharmacological approach. Endothelial activation was assessed by monitoring adhesion molecule expression, using flow cytometry and quantitative PCR. Leukocytes were incubated with HUVEC and leukocyte trans-endothelial migration was monitored using a colorimetric assay. A DNA-methyltransferase (DNMT) inhibitor was used to address the role of DNA hypomethylation in endothelial cell activation. Global DNA methylation was quantified using ELISA. Specific DNA methylation was measured by targeted bisulfite sequencing.

Results: Excess SAH increased the expression of several adhesion molecules, augmented leukocyte transmigration, and lessened global DNA methylation (each, at \( p<0.05 \)). DNMT inhibition reproduced the up-regulation of ICAM-1 (intercellular adhesion molecule-1; \( p<0.05 \)), suggesting that hypomethylation of the ICAM1 promoter may contribute to its up-regulation. However, detailed methylation analysis revealed that the ICAM1 promoter was fully demethylated prior to SAH accumulation or DNMT inhibition.

Discussion: Here, we show that SAH-induced hypomethylating stress leads to the up-regulation of key adhesion molecules. Our study confirms the physiological relevance of SAH-mediated endothelial cell activation showing that it favors leukocyte transmigration. We provide additional evidence that excess SAH promotes global DNA hypomethylation in endothelial cells. Importantly, the increased expression of adhesion molecules was partially replicated by the use of a specific DNA methylation inhibitor, suggesting a role for SAH-induced epigenetic alterations in homocysteine-associated pro-atherogenic endothelial responses, which appear to be independent of ICAM-1 promoter hypomethylation. Further analysis is needed to address more fully the key methylation targets altered by excess SAH.

Conflicts of interest: none.

Cerebellar Wnt-signaling pathway alterations in a methyl donor deficiency rat model

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Introduction: During the past decades, Methyl Donor Deficiency (MDD) has been described as a major health issue involved in developmental delays such as neural tube defects. Adequate methyl donor intake is crucial in periconceptional period (before and during pregnancy and during lactation) and one-carbon metabolism impairments have dramatic effects in the development of several organs and especially of the brain. Cerebellum is particularly affected and defects lead to behavioral changes in posture and motricity. Previous studies carried out in the lab showed that neurosteroidogenesis was decreased in Purkinje cells of new-born rats, the females being more affected than males by MDD. However, the molecular mechanisms underlying the cellular responses to this deprivation remain not well understood.
**Goal:** Our goal was to determine the functions and molecular pathways dysregulated in 21 d.o. female’s cerebellum exposed to MDD during gestation

**Methods:** We performed microarray analysis on 21 d.o. female’s cerebellum fed a MDD or a control diet (corresponding to the last day of cerebellum development). Misregulated genes and pathways in MDD vs controls were obtained by hierarchical classification. Differentially expressed candidate genes were further validated on other animals using qPCR and western blot.

**Results:** Numerous functions involved in cerebellum development, such as neuron growth, morphogenesis and differentiation or synaptic functions and plasticity, were downregulated following MDD. The wnt-pathway activation (which plays a key role in brain development) was affected at different levels including a decreased expression of some of key the proteins involved in this pathway, with a subsequent downregulation of target genes in female’s cerebellum at 21 d.o.

**Discussion:** MDD is known to be linked to brain development defects but the understanding of the molecular mechanisms involved must be deepened. The dysregulation of wnt-signaling pathway in cerebellum could explain several of cellular defects and behavioral changes previously observed in response to MDD.

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**P32** Shantanu Sengupta

**Vitamin B12 restriction leads to altered global methylation profile in kidney**

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**Introduction:** In utero nutritional perturbations could affect the germline and hence program the offspring for adult onset cardio-metabolic diseases. This is believed to occur via epigenetic mechanism like DNA methylation. Deficiency of vitamin B12, a co-factor necessary for the conversion of homocysteine to methionine in the one carbon metabolism is one such micronutrient, in utero deficiency of which could lead to predisposition of the offspring towards cardio-metabolic disorders.

**Aim:** To identify differentially methylated regions in kidney tissue of pups born to mothers fed with vitamin B12 restricted diet and to check whether these changes revert on rehabilitation at conception.

**Methods:** Methylated DNA immunoprecipitation sequencing (MeDIP-Seq) was performed in kidney tissue of pups. A total of 12 samples (6 males and 6 females) were sequenced. After initial quality control and trimming of the data, sequencing reads were mapped onto rn6 genome using Bowtie2 pipeline. MACS 1.4 algorithm was used for genome wide methylated peak identification.

**Results:** Correlation between the two replicates was around 90%. A total of 14,612 regions were hypermethylated and 3838 regions were hypomethylated in kidney of pups born to mothers fed vitamin B12 deficient diet. Most of these were reverted to control levels on rehabilitation with vitamin B12 at conception. These regions were enriched in important biological pathways like PPAR signalling, calcium signalling, fatty acid metabolism and PI3K signalling. Hypomethylation was observed in the promoters of genes like FTO, KCNQ1, PDE7B all of which belong to diabetes susceptibility loci. Additionally altered methylation was observed in CYP4A11, CYP4A22 genes which are related to renovascular hypertension.

**Discussion:** Vitamin B12 deficiency is related to changes in methylation at several sites of the genome particularly promoters, 5'UTRs, 3'UTRs and exons. Promoter regions of several genes involved in diabetes induced nephropathy and hypertension were found to be differentially methylated.

**Conflicts of interest:** None Declared.

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**P33** Kourosh R. Ahmadi

**The consequences of one Carbon Metabolism on site-specific DNA Methylation**
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**Introduction:** One-carbon metabolism provides a direct link between dietary folate/vitamin B12 exposure, the activity of the enzyme methylenetetrahydrofolate reductase and epigenetic regulation of the genome via DNA methylation.

**Aim:** A previous study by Friso et al. (2002) has shown that the common c.677C>T polymorphism in MTHFR influences global DNA methylation status through a direct interaction with folate or homocysteine (Hcy) levels. To build on this observation, we investigated whether interaction between mildly elevated plasma homocysteine and the c.677C>T polymorphism is associated with epigenome-wide, site-specific changes in DNA methylation in humans.

**Methods:** We used data on plasma Hcy levels, c.677C>T polymorphism, and site-specific DNA methylation levels (Illumina 450k array) from 1,320 individuals from the TwinsUK (n=630) and Rotterdam study (n=690). We carried out methylome-wide association studies in each cohort, modelling the interaction between levels of Hcy, c.677C>T genotypes and site-specific DNA methylation beta values, followed by a meta-analysis of the results from each cohorts. Finally, we used data from BIOS QTL browser to determine whether the identified DM sites affect the level of expression of the cognate or nearby genes.

**Results:** Our meta-analysis identified a total of 13 probes at which site-specific DNA methylation was significantly associated with [c.677C>T x Hcy] levels in both cohorts (FDR= 0.03 - 1.21E-04). The most significant associations were with a cluster of probes at the AGTRAP-MTHFR-NPPA/B gene locus on chromosome 1 (FDR=1.27E-04). Our meta-analyses identified additional probes on chromosomes 2, 3, 4, 7, 12 and 19. Interestingly, our top hit on chromosome 1 was functionally associated with variability in expression of the TNFRSF8 gene/locus on this chromosome.

**Discussion:** This is the first study to provide a direct link between perturbations in 1-CM, through an interaction of dietary folate/vitamin B12 exposure and the activity of MTHFR enzyme, and epigenetic regulation of the genome via DNA methylation.

**Conflicts of interest:** None.

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**P34 Adrian McCann**  
**Investigation of Plasma Homocysteine and Glycine in relation to Cardiovascular and Non-Cardiovascular Mortality**

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**Introduction:** Homocysteine and glycine are metabolically linked, primarily through one-carbon unit generating reactions. Elevated total homocysteine (tHcy) is associated with low levels of folate, vitamins B12 and B6, as well as impaired renal function. High levels of Hcy are linked to increased risk of atherosclerotic cardiovascular disease and its complications, however, much controversy surrounds the use of tHcy as a risk factor for cardiovascular related conditions and mortality.

**Methods:** We previously investigated 4155 (72% male) participants undergoing coronary angiography for suspected stable angina pectoris (SAP) and observed that plasma glycine was inversely related to an adverse cardiometabolic risk profile, previous AMI, more extensive CAD at angiography, and more prevalent use of CVD medications (all P<0.001). We observed that B-vitamins, and somewhat unexpectedly, tHcy also increased with increasing glycine quartiles (Table 1).
Based on the above observations we examined tHcy and glycine as mutual modifiers of Hcy-related and glycine-related risk. To evaluate the risk of all-cause mortality, cardiovascular, and non-cardiovascular death we used cox regression with additional adjustment for age, gender, renal function (creatinine), and B-vitamin intervention. Results are reported as hazard ratio (HR) per 1 standard deviation of the predictor.

**Results:** High plasma tHcy was associated with increased risk of mortality (HR 1.27, P<0.001), cardiovascular (HR 1.28, P<0.001), and non-cardiovascular (HR 1.26, P<0.001) death. Notably, these relations were strengthened after adjustment for glycine. Conversely, high plasma glycine was associated with decreased risk of mortality (HR 0.82, P<0.001), cardiovascular (HR 0.80, P<0.001), and non-cardiovascular (HR 0.85, P<0.001) death, and again, risk relations were strengthened after adjustment for Hcy.

**Conclusion:** In the present investigation, higher plasma tHcy was associated with increased risk of all-cause, cardiovascular and non-cardiovascular mortality while higher plasma glycine levels was associated with decreased risk. The data indicates the possible existence of metabolic interactions where the effects of homocysteine may be influenced by glycine, and vice versa.

**Keywords:** homocysteine – glycine – cardiovascular – non-cardiovascular – mortality – plasma
Lower Urine Glycine Concentrations Are Associated With Incident Atrial Fibrillation


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Introduction: Glycine is vital for a number of biological reactions, and lower plasma concentrations are associated with an adverse cardiometabolic risk profile. However, the association between systemic and urinary glycine levels with atrial fibrillation (AF) is unknown.

Aim: We investigated cross-sectional and long-term prospective relationships between plasma and urine glycine with AF among patients evaluated for stable angina.

Methods: Information on plasma and urine glycine concentrations was available among 4155 and 3718 patients, respectively. Between-group differences were assessed with Mann-Whitney U-test. Information on incident AF was obtained from Norwegian national health registries, and the prospective relationships explored by Cox proportional hazard modeling.

Results: Overall median (25-75 percentile) plasma and urine glycine concentrations were 205 (178-243) μmol/L and 69.3 (49.7-99.0) mmol/mol creatinine, respectively, and lower among patients with previous AF than those without (199 (171-234) vs. 206 (179-245) μmol/L; P=0.002, and 65.8 (48.3-91.2) vs. 69.6 (49.9-100.0) mmol/mol creatinine, respectively; P=0.04).

In the subset of patients without previous AF, 411 patients (10.8%) were reported with new-onset AF after median 7.3 years of follow-up. There was no association between plasma glycine and incident AF (HR (95% CI) 0.92 (0.83-1.02); P=0.98.). The crude HR (95% CI) for incident AF per 1 SD log-transformed urine glycine was 0.83 (0.75-0.92); P=0.001). Similar estimates were observed after adjusting for age and gender (0.84 (0.75-0.93); P=0.001), as well as further controlling for diabetes, smoking, hypertension, BMI, or the use of loop diuretics (0.86 (0.78-0.97); P=0.01). Adding urine glycine yielded NRI>0 (95% CI) 11.0 (0.01-22.0); P=0.04.

Discussion: Patients with AF have lower plasma and urine glycine concentrations than patients without AF. Glycine in urine, but not in plasma, was inversely related to and improved risk prediction of incident AF among patients with suspected angina pectoris.

Conflicts of interest: None

Plasma cystathionine is associated with increased risk of acute myocardial infarction: results from two independent cohort studies

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Introduction: Cystathionine is a thio-ether containing amino acid, and its plasma levels are reported to be elevated under numerous pathological conditions, including heart disease, diabetes, and renal failure. Further, cystathionine is redox-active, and data from clinical studies suggest that plasma cystathionine may be associated with vascular toxicity.

Aim: To evaluate plasma cystathionine levels in relation to acute myocardial infarction (AMI) among patients with suspected and/or verified coronary heart disease (CHD)

Methods: Subjects from two independent cohort studies, the Western Norway Coronary Angiography Cohort (3033 patients with suspected stable angina pectoris (SAP); 263 events within 4.8 y of follow-up) and the Norwegian Vitamin Trial (3670 patients with AMI; 683 events within 3.2 y of follow up) were included.
Results: In both studies, plasma cystathionine was associated with several traditional CHD risk factors (P<0.001). Comparing the highest cystathionine quartile to the lowest, age and gender adjusted hazard ratios (95% confidence intervals) for AMI were 2.08 (1.43–3.03) and 1.41 (1.12–1.76) among patients with SAP, and AMI, respectively. Additional adjustment for traditional CHD risk factors slightly attenuated the risk estimates, which were generally stronger among patients with higher median age, lower body mass index or eGFR (Pinteraction≤0.04); however B-vitamin intervention did not introduce any significant effect modifications (Pinteraction≥0.15). Further, in a subset SAP cohort, cystathionine correlated strongly negatively with glutathione, the ubiquitous antioxidant (r=-0.45; P<0.001) and positively with lanthionine, marker of H2S production (r=0.59; P<0.001).

Discussion: The present findings, based on two prospective cohort studies, suggest that, plasma cystathionine is independently associated with increased risk of AMI among patients with CHD, and possibly related with altered redox homeostasis. Future studies are warranted to explore these relationships further and to evaluate their potential clinical implications.

Conflicts of interest: None.

P37 Shantanu Sengupta

Detailed Molecular mechanism of cysteine induced toxicity

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Introduction: In Indian Population high levels of cysteine have been shown to be associated with Vitamin B12 deficiency and cardiovascular disease (Kumar et al 2009). Hypercysteinemia is also reported to be associated with Obesity and Neurological disorders. Elevated cysteine is also reported to be toxic in various model systems. However, despite the fact that cysteine can cause toxicity and is associated with many diseases the mechanism of how excessive cysteine causes toxicity is not known.

Aim: Genetic, Proteomic and Metabolic approach to delineate the mechanism of cysteine induced toxicity using yeast as a model system


Results: Quantitative proteomics approach of iTRAQ and SWATH-MS were used to characterize the cellular response during hyper- cysteinemia. We found that Ribosomal proteins and amino acid biosynthesis proteins were up-regulated, however proteins involved in Glycolysis, TCA cycle were downregulated due to high levels of cysteine. Further after an un-biased screen for amino acids and TCA intermediates (including Pyruvate), we found that Leucine and pyruvate supplementation can rescue cysteine toxicity. Genetic screen of around 4800 non-essential genes identified genetic factors which are required for survival in high levels of cysteine. Among these genes we also identified genetic factors responsible for Leucine & Pyruvate induced rescue. Intercellular amino acid measurement using o-Phtalaldehyde based derivatization revealed that hyper-cysteinemia causes an imbalance of other amino acids. Further by using s35 radiolabelling and polysome profiling we found that high levels of cysteine induces translational arrest.

Conclusion: Excess cysteine induces an amino acid imbalance and hence decreases protein translation.

P38 Gemma Ornosa-Martin

Early pregnancy folate and cobalamin status and overweight and obesity in children at 7.5 years.

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Introduction: Early pregnancy one carbon metabolism (1CM) may be associated with the programming of adiposity and metabolic function in the offspring.

Aim: To investigate the associations between maternal folate and cobalamin status and child overweight and obesity, and plasma adiponectin.

Methods: First trimester fasting plasma folate and cobalamin (microbiological assays) and MTHFR 677C>T genotype were determined in 187 mother-child pairs from the Reus-Tarragona Birth Cohort study. Anthropometric measurements from children at 7.5 years old were recorded and plasma adiponectin determined by ELISA (Milliplex Map Plex Kit, Merck Millipore). Associations between maternal 1CM factors and child outcomes were explored by multivariate linear regression and logistic regression analyses.

Results: Multiple linear regression analysis (reporting β-coefficients for the maternal–child associations) adjusting for maternal pre-pregnancy BMI, lifestyle habits MTHFR 677CT genotype (model 1) and further in a second model, for child BMI among other factors (model 2) revealed a positive association between first trimester plasma folate and child plasma adiponectin: 0.22 (P<0.01) and 0.17 (P<0.05) in Models 1 and 2 respectively. First trimester plasma cobalamin was positively associated with child plasma cobalamin: 0.23 (P<0.05) and 0.24 (P<0.05) in Models 1 and 2 respectively. Multiple logistic regression analysis confirmed that children of mothers in the highest first trimester plasma folate tertile compared to low-mid tertiles were more likely (OR (95% CI)) to have high tertile adiponectin 3.3 (1.5, 7.5) and less likely to be overweight or obese 0.34 (0.12,0.97).

Discussion: Early pregnancy folate status is associated with adiponectin and lower likelihood of overweight and obesity but not with folate status (probably due to the transitory effect of first trimester folic acid supplements) in the child. Maternal and child cobalamin status were positively associated.

Authors have no conflicts of interest to declare. Funding: FIS (cofinanced FEDER) (10/00035, 13/02500 and 16/00506), CICYT, Spain (SAF2005-05096).

P39 Anne Parle-McDermott The impact of MTHFD1L expression on formate levels and the cellular proteome in a cell line model.

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Introduction: We previously identified the mitochondrial 10-formyltetrahydrofolate synthase enzyme, MTHFD1L, as a risk factor for human Neural Tube Defects (NTD). This genetic association was further supported by a mouse model of mutant mthfd1l that exhibited an NTD and was rescued with maternal formate supplementation. Mitochondrial one carbon metabolism has since been identified as an important driver in cancer progression with many of the enzymes, including
MTHFD1L, showing significantly increased levels of expression. MTHFD1L performs the last step in mitochondrial one carbon metabolism to produce formate for transport into the cytoplasm.

**Aim:** Given the pivotal role of MTHFD1L in human disease, we sought to decipher the cellular response to the expression level of MTHFD1L in HEK293 cells.

**Methods:** Human MTHFD1L was overexpressed in a stably transfected line using a pcDNA3.2 vector and knocked down using two inducible shRNA constructs that were clonally selected. Cells were grown and sampled over a five-day period. Expression level was confirmed by RT-qPCR. Intracellular and media formate levels were measured using GC-MS. Proteomics analysis was performed on whole cell lysates using LC-MS/MS on an Ultimate 3000 nano LC system coupled to a LTQ Orbitrap XL.

**Results:** Intracellular and media formate levels directly correlated with expression level of MTHFD1L compared to controls within an approximately 1.5 to 3 fold range. Our proteomics analysis showed that MTHFD1L expression level had an effect on proteins involved in DNA synthesis, replication and repair.

**Discussion:** We have demonstrated that MTHFD1L expression level has a direct impact on both intra- and extra-cellular levels of formate and may act as a signal for uncontrolled cell proliferation. This points toward the importance of formate status in human disease and supports utilizing circulating formate levels as a more informative measure of mitochondrial folate one carbon metabolism and possibly disease status.

There are no conflicts of interest.

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**P40 Methionine demethylation and protein damage: time-dependent accumulation of homocysteine in human hair keratin**

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**Introduction:** Although homocysteine (Hcy) is a non-coded amino acid, proteins carry Hcy residues linked via an isopeptide bond to lysine residues or via a disulfide bond to Cys residues (Jakubowski H. Homocysteine in Protein Structure/Function and Human Disease. http://www.springer.com/us/book/9783709114094). Hcy is also likely to occur in proteins at positions normally occupied by methionine (Met) due to iron- or copper-dependent Met->Hcy demethylation reaction.

**Aim:** To examine a hypothesis that Met demethylation occurs during aging of long-lived proteins and leads to protein damage.

**Methods:** Keratin was extracted with SDS/DTT at 65°C from 3-cm sections along the human hair shaft. Hcy and Met were quantified by HPLC. Keratin damage was assessed by solubility.

**Results:** We found that Hcy-keratin increased linearly along the human hair shaft and was proportional to hair shaft section age (**Figure**). Young hair (≤3 months old, 0-3 cm from the scalp, n=11) contained 74.7±28.0 pmol N-Hcy/mg hair (34.3 %) and 137.5±20.2 pmol S-Hcy /mg hair (66.7%). In old hair (39-42 months old, 39-42 cm from the scalp, n=11) N-Hcy increased to 326±161 pmol/mg hair (78.6%) (p=0.003) and SHcy decreased to 74.4±37.0 pmol/mg hair (21.4%) (p=0.003). Met content in old hair keratin was significantly reduced relative to young hair keratin (20.8±3.3 vs. 25.1±3.9 μmol/mg hair, p=0.011). Model experiments show that Met was demethylated to Hcy by Fe+2 and Cu+1, which arise via reduction of Fe+3 and Cu+2, respectively, with ascorbic acid, a common component of shampoos. In old hair only 8.6±8.8 % Hcy-keratin was SDS/DTT soluble, compared to 36.6±18.3 % in young hair (p=0.0002).

**Discussion:** Our findings show that damaged, SDS/DTT-insoluble N-Hcy-keratin accumulates during
aging of head hair. Our data suggest that N-Hcy in keratin is produced by iron or copper-dependent demethylation of Met residues, which most likely occurs during washing with ascorbic acid-containing shampoos.

**Conflicts of interest:** The authors declare no conflict of interest. Supported by NCN grants 2012/07/B/NZ7/01178, 2013/09/B/NZ5/02794, 2013/11/B/NZ1/00091, 2013/09/D/ST5/03909.

**P41 Yvonne Lamers** Reference interval for serum methylmalonic acid concentration as functional biomarker for vitamin B-12 deficiency in children and adolescents

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**Introduction:** Nutritional vitamin B-12 (B-12) deficiency in children and mild inborn errors of metabolism can lead to detrimental and life-long health consequences. Both conditions present with non-specific clinical symptoms, making it crucial to rely on biomarker for their early diagnosis. Screening for B-12 deficiency is challenged by the lack of age-specific cutoffs for B-12 biomarkers, including for the functional indicator methylmalonic acid (MMA).

**Aim:** To describe the age- and sex-specific distribution of circulating serum MMA concentration and calculate a reference interval for serum MMA in children and adolescents.

**Methods:** Serum samples of 338 participants, aged 2 days to 18 years, of the Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) Project were analyzed for MMA concentrations using LC-ESI MS/MS (inter-assay CV <5.0%). The CALIPER Project aims to establish a database of reference intervals for biochemical indicators in children and to describe the influence of ethnicity, age, sex, and body mass index on biomarker concentrations.

**Results:** Serum MMA concentration did not differ between males and females (49%) across all age groups (Mann-Whitney-U-test; P>0.17; Figure 1). The age partitions 0–<1 year and 1–18 years were significantly different applying the Harris & Boyd method. We thus calculated reference intervals for these two age ranges, according to clinical guidelines (CLSI EP28-A3c). After Box-Cox transformation to achieve normality and removal of outliers after Tukey, the reference interval (2.5th–97.5th percentile) for serum MMA concentration was 111–1295 nmol/L in infants aged 0–<1 year (n=125) and 91–398 nmol/L in children aged 1–18 years (n=209).

**Discussion:** This is the first study to provide a reference interval for serum MMA concentration, the most specific functional indicator of nutritional and metabolic B-12 deficiency, in the youngest and most vulnerable population group to adverse health outcomes related to B-12 deficiency.

**Conflict of Interest:** none to declare

**P42 Deirdre O'Connor** Variations in vitamin B12 and folate concentrations in a population: implications for cognitive function? Findings from The Irish Longitudinal Study on Ageing (TILDA).

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Current evidence suggests that an imbalance of low vitamin B12 (cobalamin) status and high folate status may be associated with negative health outcomes in older adults and children (1). Moreover, it has been indicated that high folate status or the use of folic acid supplements may have a negative effect on cognitive function in older people with low vitamin B12 concentrations (2). The aim of this study was to establish the prevalence of vitamin B12:folate categories of older adults and examine the relationship with cognitive function, using data from Wave 1 (2009-2011) of The Irish Longitudinal Study on Ageing (TILDA), a nationally representative cohort of community-dwelling adults aged 50 and over (n=8175).

Blood samples were analysed for concentrations of B12 and plasma folate (n=3,914). Four categories were defined: **low B12/normal folate** (total cobalamin <= 258 pmol/L, plasma folate <=45.3 nmol/L), **low B12/high folate** (total cobalamin <=258 pmol/L, plasma folate >45.3 nmol/L), **high B12/normal folate** (total cobalamin >=258 pmol/L, plasma folate <=45.3 nmol/L), **high B12/high folate** (total cobalamin >=258 pmol/L, plasma folate >45.3 nmol/L).
normal B12/normal folate (total cobalamin >258 pmol/L, plasma folate <=45.3 nmol/L) and normal B12/high folate (total cobalamin >258 pmol/L, plasma folate >45.3 nmol/L). The Mini-Mental State Examination (MMSE) was used to assess global cognitive performance (3). Multiple regression analyses were used to examine relationship of each category with global cognitive function. The mean (SD) age of the population was 63.8 years (10.3), 48.5% were female. The prevalence of normal B12/normal folate status was 55.0% [53.2-56.8]; low B12/normal folate status was 38.5% [36.8-40.2]; low B12/high folate status was 1.8% [1.4-2.4] and normal B12/high folate status 4.6% [3.8-5.6]. Mean MMSE scores for variations in B12/folate status were not significantly different from one another after adjustment for covariates (Figure 1).

**Figure 1. Mean MMSE Score by vitamin B12/folate status category**

Mean MMSE scores for the four categories of vitamin B12 and folate status in the Irish population*: Normal B12/Normal Folate 28.1 [28.0-28.2]; Low B12/Normal Folate 27.8 [27.6-28.0]; Low B12/High Folate 27.8 [27.1-28.5] and Normal B12/High Folate 28.1 [27.6-28.6]

*covariates: age, gender, body mass index, educational attainment, depressive symptoms and disability.

Previous evidence has indicated that low B12 and high folate status was associated with negative health outcomes in older adults, particularly cognitive function. Our data does not support this hypothesis, however we plan to investigate this interaction further with a suite of in-depth cognitive tools and a higher threshold for folate status. These findings may have implications for future policy recommendations in Ireland, where a voluntary folic acid fortification programme is currently in place.


**Acknowledgements:** Funding to conduct this work was provided by the Irish Department of Agriculture, Food and the Marine through the grant 13F492 the Nutritional Biomarker Database Enhancement Initiative of ("BIO-TILDA" 2013-2015). Original funding for the TILDA study was provided by The Atlantic Philanthropies, Irish Life plc. and the Irish Government.

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**P43 Elisabet Söderström**

**Effect of Plasma Cotinine Concentration on Homocysteine Concentrations in Smokers and Users of Smokeless Tobacco**

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**Introduction:** Plasma total homocysteine (tHcy) is a risk marker for cardiovascular disease, while smoking is an established risk factor. Cotinine in plasma is a biomarker for nicotine exposure. Moist smokeless tobacco (snus) is a high nicotine containing tobacco. The effect of snus on plasma homocysteine has not been investigated so far.

**Aim:** The primary aim of this study was to investigate if smoking had a stronger impact on tHcy concentrations than the smokeless tobacco product snus after adjustment for potential confounders. We also wanted to explore whether plasma cotinine concentrations were a better predictor of tHcy concentrations than self-reported data on smoking and snus use.

**Methods:** We studied a cross-section of subjects from Northern Sweden. Tobacco exposure was
assessed by self-reported smoking and snus use, and measurement of plasma cotinine concentrations. Plasma concentrations of tHcy, creatinine, folate, B12 as well as methylenetetrahydrofolate reductase (MTHFR) 677C>T genotype were also analyzed. The study included 194 current smokers (never snus users), 47 snus users (never smokers), and 540 non-users of tobacco.

**Results:** Cotinine was positively associated with tHcy among smokers but not in snus users despite higher cotinine levels in this group. Including the MTHFR polymorphism in the analysis showed a previously not described interaction effect of cotinine by MTHFR 677C>T on tHcy concentrations. In this model, cotinine was negatively associated with tHcy in snus users. No association was seen between tHcy and number of cigarettes/day.

**Discussion:** The positive association between cotinine and tHcy in smokers but not among snus users indicates that substances other than nicotine in tobacco smoke could be responsible for the differential effects on homocysteine status, including an interaction with the MTHFR 677C>T polymorphism. Self-reported smoking should be complemented by cotinine assay whenever possible.

**COI:** Dr. Ueland is a member of the steering board of the nonprofit Foundation to Promote Research into Functional Vitamin B12 Deficiency, which owns Bevital, the company that carried out biochemical analyses. The other authors declare that they have no competing interests. This work was supported by Swedish Research Council for Health, Working Life and Welfare (former Swedish Council for Working Life and Social Research, Dnr 2007-0925 to IJ) and the Northern Counties Council. The funding body had no role in conducting this study or in writing this report.

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**P44**

**Establishing reference intervals for serum ferritin and vitamin B12 using a modified Hoffmann’s approach**

Agata Sobczyńska-Malefora, Martin A Crook, Dominic J Harrington, Alexander Katayev.

**Introduction:** Age and/or sex specific population-based reference intervals (RIs) are rarely available or difficult to establish in Clinical Laboratories. With an increased focus on the between-method standardization/harmonization of test results, the development of universal RIs for standardized/harmonized assays may help laboratories to improve patient care. Both serum ferritin and serum vitamin B12 (B12) concentrations vary with age and gender, yet unified RIs are often applied. Both lower and upper limits for these markers are clinically important, since low values suggest deficiency leading to anaemia, and high values may reflect iron overloading/acute phase (ferritin) or abnormalities in vitamin B12 binding proteins e.g. as seen with some cancers (B12). Therefore, accurate and subgroup-specific RIs should be applied.

**Aim:** To establish RIs for ferritin and B12 using a modified Hoffmann’s approach.

**Methods:** All ferritin results processed between Aug 2014-Jul 2015 and B12 processed between Jan-June 2013 on i2000SR (Abbott Diagnostics) from a population served by Guy’s and St. Thomas’ Hospitals in London, UK were used to calculate RIs. Data was partitioned in accordance with literature based knowledge about sex/age related differences in these markers.

**Results:** The RIs with percentage of values below and above the cut-offs are shown in Table 1. Because of low sample numbers, separate RIs for age group 0-12 months were not calculated.

**Discussion:** The RIs for serum ferritin and B12, calculated using a modified Hoffmann’s approach are consistent with RIs established using harmonized methods and may serve as universal RIs for other laboratories using the same methodology. They incorporate variations related to age, gender, method and the population being tested. The variations in upper limits for ferritin are of particular interest in view of iron overloading and deserve further investigations. Application of these RIs can assist with a better assessment of iron and vitamin B12 status.
Conflict of interest: All authors declare no conflict of interest.

<table>
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<th>% above upper limit</th>
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P45 Ida V. D. Schwartz

The burden of consanguinity: CBS deficiency and two other Mendelian recessive disorders

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Introduction: Classical homocystinuria (cystathionine β-synthase, CBS deficiency) can be misdiagnosed specially in patients with atypical or attenuated phenotypes.

We report a CBS deficient adult patient first diagnosed as Juvenile Idiopathic Arthritis (JIA). Case report: male, 33 yo, son of first-cousin couple, showing normal psychomotor development. At 3 mo, hand contractures were noticed. At 6 y, he received the diagnosis of JIA due to multiples contractures and articular pain. At that time, methotrexate and corticosteroids were prescribed. Hearing loss was diagnosed at age of 9. Bilateral total hip arthroplasty due to femoral head necrosis was performed at age of 20 and surgery for ectopia lens at 23. He began psychiatric care at with 31 y due to auditory hallucinations and was then referred for genetic evaluation, receiving olanzapine and escitalopram daily and methotrexate 25 mg once a week. His serum total homocysteine (tHcy) was of 431 umol/L (RV: 5-15) and of methionine of 42umol/L (RV: 13-37). Sanger sequencing of CBS gene showed homozygosity for p.Ile278Thr. He started 600 mg of pyridoxine and tHcy decreased to 31 umol/L. Methotrexate was discontinued for 7 months, but restarted because of aggravation of articular pain. He is currently on pyridoxine, folic acid, calcium, alendronate, antipsychotics and methotrexate 25 mg once a week. His serum total homocysteine (tHcy) was of 431 umol/L (RV: 5-15) and of methionine of 42umol/L (RV: 13-37). Sanger sequencing of CBS gene showed homozygosity for p.Ile278Thr. He started 600 mg of pyridoxine and tHcy decreased to 31 umol/L. Methotrexate was discontinued for 7 months, but restarted because of aggravation of articular pain. He is currently on pyridoxine, folic acid, calcium, alendronate, antipsychotics and methotrexate. His level of tHcy is currently of 17.62 umol/L. Because CBS deficiency is not expected to cause arthritis, femoral head necrosis and hearing loss, whole exome sequencing was performed. Diagnosis of CBS deficiency was confirmed and 2 other Mendelian autosomal recessive conditions were identified: nonsyndromic AR deafness (homozygosity for p.Ala138Glu in TMPRSS5) and Camptodactyly-Arthropathy-Coxa Vara-Pericarditis Syndrome (CACP) (homozygosity for p.Lys1253* in PRG4).

Discussion/Conclusion: CACP arthropathy is associated with camptodactily and head femoral necrosis, and must be distinguished from JIA, which requires anti-inflammatory drugs. This case shows the importance of additional investigation in cases of CBS deficiency with symptoms that cannot be explained by CBS deficiency alone, specially in consanguineous families.
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Classical homocystinuria (cystathionine β-synthase deficiency, CBS) is an inborn error of amino acid metabolism that requires lifelong follow-up and intervention, which may affect Quality of Life (QoL).

Objective: To evaluate the QoL of patients with CBS deficiency.

Methods: Eleven LAT CBS deficient patients were included in the study (Pyridoxine non-responsive= 9, Pyridoxine-responsive= 2). QoL was evaluated through the Whoqol - Bref Questionnaire. This instrument comprises 26 items, which measure the following domains: physical, psychological, social, and environment. It is self applicable in adulthood. The score minimum is zero and maximum is 100 (the best score).

Results: The mean total score found in Pyridoxine-responsive patients on treatment (n=2) was 62.7, being higher in the psychological (mean= 66.6) and smaller in the physical domain (mean= 60.7). For non Pyridoxine-responsive patients on treatment (n=7), the mean total score was 61, being higher in the social (63.8) and smaller in the psychological domains (57.4). Two female adult patients (probably non-responsive ones) filled out the questionnaire before starting treatment; the mean total score was 57.

Discussion/conclusion: Although the sample size is small, our data suggest that late diagnosed Pyridoxine-responsive patients differ in QoL regarding Non Pyridoxine- responsive patients, and that treatment may affect the scores found.

P47 Ida V. D. Crystalline Subluxation and Other Ophthalmic Findings in Patients with CBS deficiency

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Introduction: Classical homocystinuria (cystathionine β-synthase deficiency, CBS) is a rare genetic disease characterized by developmental delay, intellectual disability, ophthalmologic findings, skeletal abnormalities, high tHcys levels and thromboembolism.

Aims: To describe the ophthalmological findings found in a cohort of patients with CBS deficiency.

Methods: Clinical ophthalmologic examination and description of previous lens surgery were performed in 20 eyes of 10 patients (mean age: 26 years old; male: 7) with late diagnosis of CBS deficiency.

Results: Fifty percent of the eyes presented visual acuity ≥ 20/40, 35% from 20/50 to 20/200 and 15% <20/400. Twenty five percent presented with high myopia, 20% moderate myopia, 15% cataract, 15% glaucoma, 10% peripapillary atrophy and rarefaction of retinal pigment epithelium, 10% strabismus, 5% phthisis and 5% keratoconus. Sixty-five percent of eyes had previous surgery, 40% were facetectomies without intraocular lens implantation (IOL), 10% facectomy with posterior chamber IOL, 10% facectomy with anterior chamber IOL and 5% facectomy with scleral fixation of IOL.

Discussion/Conclusions: There are few reports in the literature describing the ocular findings associated with CBS deficiency. The position of the crystalline subluxation was surprisingly variable and can make difficult the clinically and ophthalmologically differential diagnosis with Marfan syndrome. Several postoperative findings are also described.
**P48  Linda S. Kornerup**

**Tissue distribution of oral vitamin B12 is influenced by B12 status and B12 form. An experimental study in rats**

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**Purpose:** Hydroxocobalamin (HOCbl) is the dominating Cbl form in food, whereas cyanocobalamin (CNCbl) is common in vitamin pills and oral supplements. This study compares single dose absorption and distribution of oral HO[57Co]Cbl and CN[57Co]Cbl in Cbl-deficient and normal rats.

**Methods:** Male Wistar rats (7 weeks) were fed a 14-day diet with (n = 15) or without (n = 15) Cbl. We compared the uptakes of HO[57Co]Cbl (free or bound to bovine transcobalamin) and free CN[57Co]Cbl administered by gastric gavage (n = 5 in each diet group). Rats were sacrificed after 24 hours. Blood, liver, kidney, brain, heart, spleen, intestines, skeletal muscle, 24-hour urine and faeces were collected, and the content of [57Co]Cbl was measured. Endogenous Cbl in tissues and plasma was analysed by routine methods.

**Results:** Mean endogenous plasma-Cbl was 7-fold lower in deficient vs. normal rats (190 vs. 1330 pmol/L, p <0.0001). Cbl depletion increased endogenous Cbl ratios (tissue/plasma = \( kin/kout \)) in all organs except for the kidney, where the ratio decreased considerably. Twenty-four-hour accumulation of labelled Cbl showed: HOCbl > CNCbl (liver) and CNCbl > HOCbl (brain, muscle and plasma).

**Conclusions:** The Cbl status of rats and the administered Cbl form influence 24-hour Cbl accumulation in tissues and plasma.

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**P49  John T. Brosnan**

**The partitioning of mitochondrial 10-formyltetrahydrofolate metabolism between formate and CO2**

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*Corresponding/presenting author

**Introduction:** Vitamin B12, a co-factor for methionine synthase, plays an important role in folate-dependent remethylation of homocysteine. We have shown that cobalamin deficiency results in a marked elevation in plasma formate.

**Aim:** To determine why formate accumulates in Vitamin B12 deficiency

**Methods:** De novo production of formate in rats was determined as described by Morrow et al (JBC 2015). Formate production from one-carbon precursors by isolated rat liver mitochondria was measured by GC-MS. 14CO2 production from 3-[14C]-serine by isolated rat liver mitochondria, expression of key genes (RT-qPCR) and changes in protein expression (immunoblot) were also determined.

**Results:** Vitamin B12-deficient rats (B12-def) showed a 55% increase in endogenous formate production, consistent with the elevated formate levels. Formate production by isolated mitochondria from serine, dimethylglycine, and sarcosine was increased by at least 60% in B12-def animals while 14CO2 production from serine by these mitochondria was 26% lower compared to replete controls. The expression of Mthfd1l in B12-def animals was unchanged but the expression of Aldh1l1 and Aldh1l2 was reduced by 40-60% of that in the livers of control animals.

**Discussion:** We suggest that the partitioning of mitochondrial formyl-THF between oxidation to CO2 (by ALDH1L1) and production of free formate (by MTHFD1L) is a key control point in one-carbon metabolism. In B12-def the decreased flux through ALDH1L2, in the face of a constant rate of 10-formyl-THF disposition can explain the increased production and increased concentration of formate. We speculate that the decreased hepatic SAM levels found in B12-def animals may cause increased production of one-carbon groups in the form of formate to replenish hepatic SAM. Since mitochondrial formate becomes available to the cytosol we suggest that this metabolic bifurcation may regulate the entry of formate into the cytosolic one-carbon pool.

**We have no conflict of interests to disclose**
En-Pei Chiang.

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Glycine-N methyltransferase (GNMT) is a major hepatic enzyme that converts S-adenosylmethionine to S-adenosylhomocysteine while generating sarcosine from glycine. The metabolic role of GNMT in folate-mediated one-carbon metabolism and how glycine supply may affect one carbon metabolic kinetics are under investigated in the present study. Hepatocyte-derived cell-lines with and without GNMT expression were cultured in minimum essential medium (MEM) with and without high glycine supply. Methylene-tetrahydrofolate (mTHF) utilization and endogenous formate biosynthesis were carefully investigated using stable isotopic tracers and GC-MS. We discovered that high glycine inhibits the M+1 specie enrichments in deoxythymidine (dT+1) from 2,3,3 D3-serine, suggesting that high glycine may inhibit endogenous formate production in these *in vitro* models. In a parallel experiment that exogenous formate was used as the 1C source, enrichments in dT+1 significantly increased from exogenous formate, supporting our hypothesis that high glycine inhibits endogenous formate production for thymidine synthesis. Mice fed in amino acid based diet supplemented with high glycine had significantly lower plasma formate concentrations that provide in vivo evidence on our hypothesis. On the other hand, high glycine promotes M+1 specie enrichments in methionine from 2,3,3 D3-serine, indicating that high glycine supply may favor the utilization of methyleneTHF derived one carbon for homocysteine remethylation. High glycine supply can promote folate dependent methionine synthesis in our cell models. Preliminary *in vivo* experiments demonstrated that high dietary glycine alters folate dependent methionine synthesis, consistent with our *in vitro* findings. We demonstrated how glycine supply regulates folate-mediated one-carbon metabolic kinetics *in vitro* and *in vivo*. (supported by MOST 105-2320-B005-010-MY3; MOST104-2911-I005-301; and Ministry of Education, Taiwan, R.O.C. under the ATU plan)
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