Organisation Structure and Committees

8th ISNN Organisation Committee
- Michael Fenech (CSIRO, ISNN) – President of 8th ISNN
- Lynn Ferguson (Nutrigenomics NZ, ISNN) – Chair Scientific Programme
- Peter Howe (NSA) – Deputy Chair Scientific Programme
- Artemis Simopoulos (ISNN) – Founding President of ISNN
- Raffaele De Caterina (ISNN) – Current President of ISNN
- Kate Stewart (ACNEM)
- Lynne Cobiac (CSIRO)
- Malcolm Riley (NSA)
- Barbara Meyer (NSA)
- Alison Coates (NSA)
- Boon Yee (ILSI SEA)

Supporting Organisation Acronyms
ACNEM Australian College of Nutritional and Environmental Medicine
CSIRO Commonwealth Scientific and Industrial Research Organisation (Australia)
NSA Nutrition Society of Australia
ILSI SEA International Life Sciences Institute - South East Asia
ISNN International Society of Nutrigenetics/Nutrigenomics

8th ISNN Scientific Programme Committee
Chair
Lynn Ferguson (ASIA PACIFIC CONGRESS OF NUTRITION, NUTRIGENOMICS NEW ZEALAND representative)

Deputy-Chair
Peter Howe (Joint Editor-in-Chief, Nutrients, & Fellow of the Nutrition Society of Australia)

Members
- Artemis Simopoulos (ISNN)
- Raffaele De Caterina (ISNN)
- Kate Stewart (ACNEM)
- Michael Fenech (ISNN, CSIRO)
- Lynne Cobiac (CSIRO)
- Malcolm Riley (NSA)
- Barbara Meyer (NSA)
- Alison Coates (NSA)
- Boon Yee (ILSI SEA)
- Myung-Sook Choi (ISNN)
- Jim Kaput (Institute of Health Sciences, Nestle, Lausanne, Switzerland)
- John Milner (ISNN)
- Louis Perusse (ISNN)
- Ben van Ommen (NUGO, TNO)
**Program**

### Thursday, May 1

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<td>6:00-7:00 pm</td>
<td>Asia-Pacific Nutrigenetics &amp; Nutrigenomics Organisation (APNNO) foundation meeting</td>
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<td>Buffet Dinner – Speakers, ISNN Board members and Guests</td>
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### Friday, May 2, 7:30 am–6.00 pm

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<td>President, Australasian College of Nutritional and Environmental Medicine – Eugen Molodysky</td>
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<td>8:45–9:15 am</td>
<td>OPENING LECTURE</td>
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<td>Chairperson – Raffaele de Caterina</td>
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<td></td>
<td>Alfredo Martinez – Epigenetics: nutrients and obesity (30 min)</td>
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<td>9:15–11:00 am</td>
<td>EARLY LIFE EPIGENETIC EFFECTS</td>
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<td>Emma Whitelaw – The role of epigenetics in the determination of phenotype – Keynote Lecture (45 min)</td>
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<td>Chittaranjan S. Yajnik – Foetal programming of diabetes by maternal 1-C metabolism – Symposium Lecture (30 min)</td>
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<td>Margaret J. Morris – Grand-paternal obesity induces trans-generational molecular defects in F2 offspring (15 min)</td>
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<td>Safarina G. Malik – Maternal mitochondria DNA haplotype and copy number may influence the impact of prenatal nutrition on fetal growth in Indonesia (15 min)</td>
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<td>11:00–11:30 am</td>
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<td>11:30 am–1:15 pm</td>
<td>BRAIN AND MENTAL HEALTH</td>
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<td>Chairperson – Young-Joon Surh</td>
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<td>Lyn Griffiths – Molecular genetics of migraine: implications for therapeutic development – Keynote Lecture (45 min)</td>
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<td>Ng Tze Pin – Brain and mental health – nutritional influences in older persons – Symposium Lecture (30 min)</td>
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<td>Philip Thomas – Buccal cytome biomarkers and their association with plasma folate, Vitamin B12 and homocysteine in Alzheimer’s disease (15 min)</td>
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<td>Sarah Brooker – The interactive effect of an Australian-type diet, micronutrient supplements and Alzheimer’s disease (AD)-prone genotype on AD-associated behaviour and neuropathology (15 min)</td>
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<td>1:15–2:30 pm</td>
<td>Lunch and Poster Session</td>
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2:30–4:30 am  EARLY CAREER SCIENTIST SESSION  Chairpersons – Matthew Barnett and Nick Hudson

Emma Beckett – Vitamin D receptor genotype modulates the correlation between circulating levels of miR-7a/b and vitamin D intake in an elderly cohort (15 min)

Sau Lai Lee – Extracellular Amyloid Beta 42 Causes Necrosis, Inhibition of Nuclear Division, and Mitotic Disruption Under Both Folate Deficient and Folate Replete Conditions as Measured by the Cytokinesis – Block Micronucleus Cytome Assay (15 min)

Caroline Bull – Folate deficiency induces dysfunctional long and short telomeres; both states are associated with hypomethylation and DNA damage in human WIL2-NS cells (15 min)

Ju-Sheng Zheng – Circulating 25-hydroxyvitamin D, IRS1 variant rs2943641 and insulin resistance: replication of a gene-nutrient interaction in four populations of different ancestries (15 min)

4:30–5:30 pm  EARLY CAREER SCIENTIST SESSION  Chairpersons – Matthew Barnett and Nick Hudson

5:30–6:00 pm  SPECIAL LECTURE  Chairperson – Michael Fenech

Ben van Ommen – N=1 nutrition research – a revolution in science and healthcare (30 min)

7:00–9:00 pm  Board Meeting of the International Society of Nutrigenetics/Nutrigenomics
M. Prakash Hande – MST-312 alters telomere dynamics, gene expression profiles and sensitises human breast cancer cells to PARP-1 inhibition (15 min)

1:30–2:30 pm Lunch and Poster Session

2:30–4:30 pm EARLY CAREER SCIENTIST SESSION Chairpersons – Matthew Barnett and Nick Hudson

Roseline Yap Wai Kuan – Association and Interaction Effect between VEGF Receptor-2 (VEGFR-2) gene polymorphisms and Dietary Pattern on blood lipids in Malaysian adults (15 min)

Cheryl S. Gammon – Kiwifruit improves the TG/HDL-cholesterol ratio in individuals with the CETP TAQ1B B1/B1 genotype (15 min)

Jean M. Winter – Diverse effects of resistant starch and red meat on proliferation and O6Methyl-2-deoxyguanosine adduct formation in the distal colon of Msh2 deficient mice: Consequences for colorectal carcinogenesis (15 min)

Zacharoula Nikolakopoulou – Omega-3 polyunsaturated fatty acids selectively inhibit growth in squamous cell carcinoma oral and epidermal keratinocytes by differentially activating ERK1/2 (15 min)

NUTRIGENOMICS TOOLS WORKSHOP Chairperson – Louis Perusse

Nick Hudson – A network science view of nutrigenomics and nutrigenetics (20 min)

Peter Molloy – Epigenomics and epigenetics (20 min)

Matthew Barnett – Proteomics & Metabolomics (20 min)

4.45–5.30 pm CLOSING CEREMONY Chairperson – Peter Howe

John Milner Commemoration
Richard Head – John Milner’s life-time achievements (15 min)

Closing Ceremony & Awards – (30 min)
The John Milner Early Career Scientist Prize (sponsored by ILSI South East Asia)
Student’s Poster Prize (sponsored by Nutrients)

7:00–11:00 pm Joint ISNN Conference and Nutrition in Medicine Conference Gala Dinner

Program information is subject to change without notice.
The word epigenetics was coined as “the study of the mechanisms of temporal and spatial control of gene activity describing pathways different from those directly attributable to the underlying DNA sequence and with an influence on the organism response”. Specific epigenetic processes include DNA methylation, histone modifications, chromatin folding or miRNA synthesis and, in general, all those phenomena eventually affecting gene expression patterns. These mechanisms together with other transcriptional regulatory events ultimately regulate gene function and expression during development or in response to nutritional and environmental stimuli.

In this context, different examples of dynamical changes in DNA methylation patterns due to the restriction or supplementation with different nutrients have been reported concerning as vitamin B6, vitamin A and some minerals. Moreover high fat/sugar intake and situations of excessive body weight are also associated with changes in DNA methylation profiles, affecting the promoter region of different genes involved in energy homeostasis and obesity such as LEP, POMC, FASN, CLOCK, and NDUFB6. Furthermore, epigenetic biomarkers are being identified in order to predict body weight maintenance after weight loss in humans, including TNF-alpha, AQP9, POMC, FASN, CLOCK, and NDUFB6. Furthermore, epigenetic biomarkers are being identified in order to predict body weight maintenance after weight loss in humans, including TNF-alpha, AQP9, ATP10A and CD44 as well as some specific miRNAs.

In summary, it is becoming evident that inter-individual differences concerning the outcomes of nutritionally-related chronic diseases such as obesity depend not only on the dietary intake and the subject’s DNA sequence, but also on the inherited epigenome and on different nutritional (intrauterine or adult) influences that modify the epigenetic marks affecting gene expression.

References

O3 – Symposium Lecture
Fetal Programming of Diabetes by Maternal 1-C Metabolism
Chittaranjan S. Yajnik
King Edward Memorial Hospital, Pune, India
Email: csyajnik@hotmail.com

Genetics has contributed little to prevention of diabetes. The most promising lead for primary prevention comes from David Barker’s ‘intrauterine fetal programming’ hypothesis. He suggested that alterations in fetal nutrition during critical stages of development permanently alter the structure and function of the fetal organs (programming).

The Pune Maternal Nutrition Study (PMNS) was established in 1993 to study the effect of maternal nutrition on the fetal growth and later risk of diabetes. Mothers were short and thin (BMI 18.1 kg/m²) and babies were small (birth weight 2.7 kg). Comparison of anthropometric measurements of these babies with those of English babies showed that these babies were thin (lean mass) but fat (higher fat mass). Maternal micronutrient-rich foods influenced fetal growth. Further measurements revealed an important influence of maternal 1-carbon metabolism on fetal growth and programming of diabetes. B12 deficiency was common but folate deficiency was rare. Maternal folate promoted, while homocysteine restricted fetal growth. Low B12 and high folate predicted higher levels of risk factors for diabetes in the child. Maternal genetic polymorphisms associated with 1-C metabolism (MTHFR) and those regulating nutrient levels (FUT-2, TCN2) predicted many of these outcomes, lending support to the idea that these associations may be causal.

These effects may act through epigenetic mechanisms (especially DNA methylation) that alter expression of genes. Substantial evidence is available in animal models, and human evidence is building up.

After demonstrating adequate absorption of oral vitamin B12 and efficacy of physiological doses (2 μg/d) we have started an intervention in adolescent girls and boys to improve their vitamin B12 and other micronutrient status to reduce the risk of adiposity and diabetes in the offspring. This trial will have important implications for future public health policy in India.

O4
Grand-Paternal Obesity Induces Transgenerational Molecular Defects in F2 Offspring
Virginie Lecomte, Christopher A. Maloney, Neil A. Youngson, Margaret J. Morris*
The University of New South Wales, Australia
Email: m.morris@unsw.edu.au

Objectives: It is now accepted that paternal obesity can contribute to offspring metabolic risk via non-genetic mechanisms, but whether transgenerational defects can be transmitted to F2 is unknown. Thus we aimed to determine the metabolic and molecular defects in grand offspring of obese male rats.

Methods: Control F1 male rats from 8 obese (ob) and 8 control (con) F0 fathers were mated with control females to generate F2 offspring. F2 animals were fed either a control diet (CD) or challenged with a high fat (HFD) diet. Tibialis anterioris muscle and liver were collected from F2 males at 26 weeks of age. Gene expression was analysed using TaqMan Real-time PCR.

Results: When F2 animals were fed HFD, increased body weight was observed in those from ob versus con F0. Liver and tibialis showed 25% increase in triglyceride content in sibling F2 fed HFD versus CD (p < 0.05, n = 8.7). Based on these results, we analysed gene expression targeting lipid metabolism pathways. In both tissues, HFD consumption in F2 from ob F0 resulted in an increased expression of genes involved in lipogenesis (e.g. Srebf) and inhibition of expression of genes involved in lipid β-oxidation (e.g. Hadh, Acat1). Genes involved in mitochondrial function (Pgc1a, Cpt1) were downregulated by grand-paternal obesity. The effect of HFD consumption in F2 interacted with grand-paternal programming, resulting in an amplification of changes in gene expression.

Conclusions: These results suggest that grand-paternal obesity disturbs key genes in lipid metabolism and mitochondrial function in muscle and liver of F2 offspring, leading to lipid accumulation in response to a HFD. This study provides the first evidence of transgenerational effects of grand-paternal obesity in the rat.
Objective: Maternal factors modulating the effects of prenatal nutrition on fetal growth are poorly understood, and may modify the impact of nutrition programs. The Supplementation with Multiple Micronutrients Intervention Trial (SUMMIT) in Indonesia (2001–2004), compared maternal multiple micronutrient (MMN) supplementation to iron and folic acid (IFA) in 32,000 women, and showed that maternal MMN decreased low birth weight (LBW) by 14%. However, heterogeneity of effects in anemic and malnourished mothers on LBW were observed. We propose a key source of this heterogeneity is the interplay between maternal nutritional status and mitochondrial DNA (mtDNA) haplotype and mtDNA copy number, thereby affecting maternal and fetal energy metabolism and birth weight.

Methods: Archived dried blood spots from a subset of SUMMIT mothers were selected consisting of 100 whose newborns were LBW and 100 with normal weight babies, and mtDNA haplotype and copy number were determined. Maternal status was classified as non-anemic and non-malnourished, anemic only, malnourished only, and anemic-malnourished. MtDNA haplotype was determined by sequencing the HVR I, and copy number by realtime PCR using the tRNA\(^{\text{Leu(UUR)}}\) as a target gene and \(\beta\)-2-microglobulin as an endogenous control. Data were analysed for associations with maternal nutrition status and low birth weight.

Results: In this ongoing study we have so far determined the mtDNA haplotype and copy number in 71 samples from mothers with LBW babies. The mtDNA haplogroups were M, E, B, F. The dominant haplogroup in anaemic and anaemic-malnourished mothers was E. MtDNA copy number was higher in anaemic and anaemic-malnourished group as compared to the other groups, suggesting the need for higher energy intake in these groups.

Conclusion: MtDNA haplogroups of mothers with LBW newborns were M, E, B and F. However, haplogroup E was dominant in anaemic and anaemic-malnourished women. Higher mtDNA copy number was observed in anemic and anemic-malnourished groups.

Brain and Mental Health

Migraine is a severe neurological disorder that affects a significant proportion of the population. Prevalence estimates for the disorder vary between 12 and 25% depending on the population studied. The disorder has a significant genetic component showing high levels of familial aggregation. Although a number of genes involved in a rare and severe sub-type of migraine, termed familial hemiplegic migraine have been identified, the number and identity of all the genes involved in the more common types of migraine have yet to be defined. Genetic linkage and GWAS studies have implicated a number of genomic regions including on chromosomes 1, 4, 11, 19 and the X chromosome and several susceptibility variants have been implicated in the disorder. Neurotransmitter pathways have been the main focus of studies investigating the molecular mechanisms of the disorder. However vascular and hormonal disturbances also occur in migraineurs, as highlighted by alterations in cerebral blood flow and hormonal triggers of migraine, particularly in women and hence factors affecting these functions may also be involved. This presentation will focus on migraine gene studies in our laboratory, including recent family and GWAS results, as well as studies implicating hormone receptor genes and MTHFR gene variants. In addition an overview of results from two recently completed clinical trials that involved genetic profiling in conjunction with a nutriceutical therapeutic will be presented. These clinical trial results are very promising and highlight the potential importance of pharmacogenetic interventions in this disorder.
buccal cell cytome biomarkers. B12 and Homocysteine levels were also measured and correlated to assess whether micronuclei and other parameters of genome damage were found to be significantly lower in Alzheimer’s patients. The odd’s ratio of being diagnosed with AD for those individuals with a combined basal and karyorrhectic cell frequency <41 per 1000 cells is 140, with a specificity of 97% and sensitivity of 82%. Homocysteine was found to be significantly increased (p = 0.0003) in the AD cohort compared to the control group. In the Alzheimer’s cohort plasma B12 showed a positive correlation with the number of micronuclei (r = 0.3552, p = 0.0425) and with the number of basal cells (r = 0.3448, p = 0.0494). Plasma Homocysteine showed a negative correlation with the number of karyorrhectic cells (r = −0.4107, p = 0.0176).

Conclusions: These cytome changes may reflect alterations in the cellular kinetics or structural profile of the buccal mucosa, and may be useful as potential biomarkers in identifying individuals with a high risk of developing AD. The study also suggests that the buccal cytome of AD subjects exhibits a different relationship to B12 and homocysteine compared to that seen in non AD controls.

09
The Interactive Effect of an Australian-Type Rodent Diet, Nutrient Supplements and Amyloid Over-Expression on AD-Associated Behavior and Neuropathology
Sarah Brooker1, *, John Power1, Michael Fenech2
1Human Physiology, Flinders University, 2CSIRO Preventative Health Flagship, Australia
Email: miss.s.brooker@gmail.com

Objectives: Alzheimer’s disease (AD) is one of the leading causes of death in Australia. Australian women are twice as likely to develop AD by 65, than Australian men. Diet is a modifiable risk factor for AD. The effect of a diet typically eaten by Australian women on the behavioural deficits and neuropathology associated with AD was assessed using an AD mouse model.

Methods: An “Australian-type” mouse diet was designed, reflecting the current nutrient intake of Australian women. A second diet was designed to investigate whether nutrient supplements could slow the progression of AD when added to the Australian-type mouse diet. Learning was assessed in the Morris Water Maze (MWM) and amyloid pathology was viewed using confocal and brightfield microscopy.

Results: AD-type mice were significantly heavier than normal mice after 25 weeks on the Australian-type diet (p = 0.01), suggesting that AD-type mice are susceptible to diet-induced obesity. This was prevented with nutrient supplements. There were no differences in body weight of AD-type mice fed the nutrient supplemented diet and normal mice.

AD-type mice fed the Australian-type diet demonstrated spatial learning in the MWM at 12 months (p < 0.001), suggesting that the...
Austalian-type diet may reduce spatial learning deficits. At 15 months, AD-type mice fed the Australian-type diet failed to demonstrate learning in the MWM, indicating that the Australian-type diet was no longer protective. 15 month old AD-type mice fed the nutrient supplements significantly improved in the MWM after five training days (p < 0.05), suggesting that nutrient supplements reduced learning impairments in AD-type mice.

Amyloid deposit size was affected by dietary macro-nutrients. AD-type mice fed the Australian-type diet, alone or with supplements, had more large (>35 um) amyloid deposits than those fed a control diet (p < 0.05). Nutrient supplementation increased the amount of amyloid associated with the blood brain barrier.

Conclusions: The effect of the Australian-type diet on AD-associated behaviour and neuropathology changed with age. The Australian-type diet conserved spatial learning abilities in adult, but not aged AD-type mice. Despite preventing obesity and learning deficits in aged AD-type mice, nutrient supplements failed to prevent diet-induced changes in pathology.

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Early Career Scientist Session

**O10**

**Vitamin D Receptor Genotype Modulates the Correlation Between Circulating Levels of miR-7a/b and Vitamin D Intake in an Elderly Cohort**

**Emma Beckett**1, 2, *, Charlotte Martin1, Konsta Duesing2, Zoe Yates3, Martin Veysey3, Mark Luccok1

1School of Environmental and Life Sciences, University of Newcastle, Australia; 2CSIRO Animal, Food and Health Sciences, Australia; 3School of Biomedical Sciences and Pharmacy, University of Newcastle, Australia; *Teaching and Research Unit, Central Coast Local Health District, Gosford, Australia

Email: emma.beckett@uon.edu.au

**Objectives:** Circulating microRNAs are linked to disease processes and have potential as biomarkers. Micronutrients can influence microRNA and gene expression via modulation of nuclear receptors, such as the vitamin D receptor (VDR), or through epigenetic mechanisms. Vitamin D status influences risk of disease, such as cardiovascular disease and cancers. Genotypic variance may influence these relationships and processes.

We examined correlations between circulating levels of two well characterised tumour-suppressor microRNAs (let-7a and -7b) and vitamin D intake, and how these relationships were modulated by two common VDR polymorphisms, Apa1 (A/a [A/C]) (rs7975232) and Bsm1 (B/b [T/C]) (rs1544410).

**Methods:** Let-7a and -7b expression were measured by qPCR in plasma (n = 200). RLFP-PCR was used for genotyping. Vitamin D intake was estimated using food frequency and supplement questionnaires. Relationships between microRNA expression and vitamin D intake were assessed using Spearman’s correlation, which were compared between genotypes using z-scores.

**Results:** Let-7b expression negatively correlated with vitamin D intake (r = –0.20, p = 0.0058). Stratification by genotype revealed that the magnitude and direction of correlation was maintained in those carrying the Bsm1 variant “b” allele (n = 176, r = –0.27, p = 0.0005). However, in the “BB” subpopulation (Bsm1 restriction site absent, n = 24), the direction of the correlation was reversed (r = +0.319, p = 0.0497), and was significantly different to the variant (“b”) allele group (z-score = 2.5, p = 0.0124). Similar results were obtained for the Apa1 variant. The only significant correlation observed between let-7a and vitamin D intake was in the subpopulation without Apa1 restriction site (AA). Neither vitamin D intake nor miRNA expression levels alone differed significantly between genotype groups.

**Conclusions:** The correlation between let-7a and -7b expression and vitamin D intake in this cohort varies with VDR genotype. While the mechanisms behind these correlations are yet to be elucidated, this study highlights the importance of considering underlying genotypic variance in microRNA expression studies, and in nutritional epigenetics generally.

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**O11**

**Extracellular Amyloid Beta 42 Causes Necrosis, Inhibition of Nuclear Division, and Mitotic Disruption Under Both Folate Deficient and Folate Replete Conditions as Measured by the Cytokinesis – Block Micronucleus Cytome Assay**

**Sau Lai Lee**, Philip Thomas, Michael Fenech

CSIRO, Preventative Health Flagship, Adelaide, Australia

Email: sau.lee@csiro.au

**Objectives:** Alzheimer’s disease is associated with accumulation of extracellular beta amyloid peptide 42 (Aβ42) which may induce DNA damage and reduce cellular regenerative potential. These effects may be exacerbated under conditions of folate deficiency. The aim of this study was to investigate whether extracellular Aβ42 induces DNA damage and cell death in human peripheral lymphocytes and whether there is an interactive effect between extracellular Aβ42 and folic acid status.

**Methods:** Peripheral blood lymphocytes were cultured in medium under conditions of both low and high folate (20 and 200 nM respectively) and challenged with either Aβ42 or the physiologically normal form Aβ40 (both at 5, 10, 15 μM). Genome stability and cytotoxicity events were investigated using the cytokinesis-block micronucleus cytome (CBMN-cyt) assay. Outcome measures scored included the nuclear division index (NDI), necrosis, apoptosis, binucleated cells with micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD) and abnormally shaped nuclei (circular, CIR and horse-shoe, HS) that may be indicative of mitotic disruption.

**Results:** Folic acid deficiency significantly reduced NDI (p < 0.001) and increased all the DNA damage biomarkers (MN, NPB,
Folate Deficiency Induces Dysfunctional Long and Short Telomeres; Both States Are Associated with Hypomethylation and DNA Damage in Human WIL2-NS Cells

Caroline F. Bull1, 2, *, Graham Mayrhofer2, Nathan J. O’Callaghan1, Amy Y. Au1, Hilda A. Picket1, 4, Grace Kah Mun Low4, Dimphy Zeegers5, M. Prakash Hande6, 6, Michael F. Fenech1

1Nutritional Genomics Laboratory, CSIRO Animal, Food and Health Sciences, Adelaide 5000, South Australia; 2Discipline of Microbiology and Immunology, School of Molecular and Biomedical Sciences, University of Adelaide, South Australia 5000; 3Children’s Medical Research Institute, Westmead, New South Wales 2145, Australia; 4Sydney Medical School, University of Sydney, New South Wales 2145, Australia; 5Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, 117597 Singapore; 6Tembusu College, National University of Singapore, 138597 Singapore

Email: caroline.bull@csiro.au

Objectives and Methods: The essential role of dietary micro-nutrients for genome stability is well documented, yet the effect of folate deficiency or excess on telomeres is not known. Accordingly, human WIL2-NS cells were maintained in medium containing 30 nM, 300 nM or 3,000 nM folic acid (FA) for 42 days to test the hypothesis that chronic folate deficiency would cause telomere shortening and dysfunction. Telomere length was measured by flow cytometry. Chromosomal instability was measured using the cytokinesis-block micronucleus cytome (CBMNcyt) assay.

Results: After 14 days, telomere length (TL) in FA-deficient (30 nM) cultures was 26% longer than that of 3,000 nM-FA cultures, however, this was followed by rapid telomere attrition over the subsequent 28 days (p-trend ≤ 0.0001); both long and short telomere status was positively correlated with biomarkers of chromosome instability (p ≤ 0.003) and mitotic dysfunction (p = 0.01), measured by the CBMNcyt assay. The early increase in TL was associated with FA-deficiency-induced global DNA hypomethylation (p = 0.05), with an effect size similar to that induced by the DNA methyltransferase inhibitor, 5-aza-2’-deoxycytidine. qPCR analysis indicated a negative association between FA concentration and uracil incorporation into telomeric DNA (r = –0.47, p = 0.1), suggesting a possible plausible mechanism for uracil as a cause of folate deficiency-induced telomere dysfunction or deletion. PNA-FISH analysis showed FA-deficiency resulted in 60% of micronuclei containing acentric terminal fragments, an observation consistent with the 3-fold increase in terminal deletions (p = 0.0001).

Conclusions: Extracellular Aβ42 appears to have cytotoxic and cytostatic effects but its effect on chromosomal instability appears to be small relative to folate deficiency.
Translation into Practice Workshop

O14
Is Nutrigenetics the Solution for Optimal Weight Management?

Louis Pérusse
Department of Kinesiology and Institute of Nutrition and Functional Foods, Laval University, Québec, Canada
Email: Louis.Perusse@kin.ulaval.ca

While a changing environment favoring increased food intake and decreased physical activity levels has clearly contributed to the rise in the prevalence of obesity observed over the past decades, not everyone is becoming overweight or obese. This suggests that there are genetic factors interacting with environmental factors to predispose some individuals to obesity. This gene-environment interaction is not only important in determining an individual’s susceptibility to obesity, but can also influence the outcome of weight loss programs and weight management strategies in overweight and obese subjects. This presentation will review the role of gene-nutrient interactions in the context of weight management, including weight loss and weight maintenance. Several candidate gene polymorphisms were shown to influence weight loss in response to lifestyle intervention involving caloric restriction, physical activity or a combination of both. Studies vary considerably in terms of design, sample size, control for confounding factors and adjustment for multiple testing, which make replication of findings very difficult. Despite overwhelming evidence suggesting that genes influence weight loss and weight maintenance following caloric restriction and/or exercise, it is still too early to consider nutrigenetics as the optimal solution for weight management.

O15
Oomics Technologies in the Clinic

Lynnette R. Ferguson
The University of Auckland, Auckland, New Zealand
Email: l.ferguson@auckland.ac.nz

Clinical trials are often costly and inefficient, partly because the most relevant end point is not necessarily known or the trial specifically designed to measure this. Thus, biomarker studies are appropriate for showing the most relevant trial end points. The most appropriate end points may not be the ones desired or selected. Omics technologies do not need to be a specific or test a single hypothesis, and they can be applied with small numbers of subjects. They can play a significant role in the development of the most appropriate design of an effective clinical trial for convincing evidence of functional food efficacy.

Subsequent clinical trials may use various – omics technologies directly as endpoints (especially transcriptomics, metabolomics and proteomics), or the technology can be used to point instead to the most effective and efficient of the standard endpoints.

Thus these techniques lead to the most efficient and effective protocols for subsequent clinical trials.

O16
Genome Health Clinics

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The ability of cells in the body to replicate and repair DNA accurately is a fundamental necessity for normal development of all organs in the body as well as regeneration of tissues. Loss of genome integrity may occur as a result of multiple causes including inherited or acquired defects in DNA maintenance, environmental genotoxic agents, life-style genotoxins, metabolic stress and micronutrient deficiencies or excesses. During the past three decades robust methods for measuring DNA damage have been developed and include cytogenetic methods such as the cytokinesis-block micronucleus assay and molecular methods that measure telomere length which have been well-validated with respect their association with exposure to environmental genotoxins, metabolic and psychological stress and life-style factors including exercise, smoking, alcohol consumption and dietary habits. These biomarkers of DNA damage and others (e.g. metaphase chromosome aberrations, DNA strand break and base damage assays) increase with age and trigger senescence or cell death leading to loss of regenerative capacity of tissues and cancer. A high level of DNA damage such as micronuclei (a biomarker of chromosome breakage or loss) and excessive telomere shortening (caused by attrition of the TTAGGG sequence at the ends of chromosomes) predict a higher risk of infertility, pregnancy complications, cancer, cardiovascular disease and neurodegenerative disease including accelerated ageing. For these reasons, the concept of Genome Health Clinics based on diagnosis and prevention of DNA damage was first developed and published seven years ago. This concept has now been translated into practice by a number of companies the first of which was Reach100 in Australia in 2007, followed by others such as Telome Health in the USA in 2009, Life Length in Europe in 2010 and Repeat Diagnostics in Canada in 2011. The presentation will explain the scope and services provided by these companies and the scientific basis of their offerings.

References and Websites:
2 Reach100. www.reach100.com.au
3 Telome Health. www.telomehealth.com
4 Life Length. www.lifelength.com
5 Repeat Diagnostics. www.repeatdiagnostics.com
Abstracts

Special Lecture

O17
N = 1 Nutrition Research: A Revolution in Science and Healthcare
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Lifestyle related diseases are ~90% preventable, but still are an epidemic threat to healthcare and health economics. So why do people not follow dietary advice? Is there something wrong with the advice? Nutrition research has come a long way and has seen a number of major developments over the last 10 years, but is nutrition science ready to “quantify” my personal health status and provide related personal dietary and lifestyle advice based on quantification of my own genotype X phenotype X environment interaction? A careful look at changes in healthcare points at the urgent need for both prevention and personal empowerment. Each individual, in order to properly take control of one’s own health, needs access or even better, needs to own all relevant information regarding his or her personal health status. Apart from the above-mentioned integrative personal omics profile, other activities point in this direction. There is a push from the “medical records” front, and more interestingly, the Quantified Self crowd source movement (http://quantifiedself.com) launches all kinds of initiatives. Developments in personal sensors are exploding. NuGO, the Nutrigenomics Organisation has taken up the challenge to initiate an open access cohort where each individual provides and owns her/his own health data in a comprehensive, “extensive phenotyping” manner, ranging from body weight to genome, and from plasma metabolome to “do-it-yourself” oral glucose tolerance test. As a first step, the “Nutrition Researcher Cohort” (NRC) is established as a 2-year project to establish all analytical methods, standards and operation procedures, data infrastructure, ethical and privacy aspects, governance, etc.). Details are provided at www.nugo.org/nrc and one can enroll at http://nrc.dbnp.org. The NRC is a “crowd science” project, where researchers, as experts/sub- jects, both participate by providing personal health data, and build by together developing analytics, bioinformatics, ethics, ICT and advice systems. Once the two year initial phase is passed, we can implement the lessons learned in a really new mix between a nutrition and health cohort and a personal healthcare setting.

CVD/Obesity/Diabetes

O18 – Keynote Lecture
The Genetic Architecture of Body-Mass-Index
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Variation in quantitative traits such as human height is caused by a combination of multiple genes and environmental effects. Since Galton in the late 1800s the genetics of such traits has been studied using concepts that refer to the combined effect of all genes (e.g., heritability), using the resemblance (or recurrence risk) between relatives. Genome-wide association studies (GWAS) facilitate the dissection of heritability into individual locus effect. They have been successful in finding many SNPs associated and have greatly increased the number of genes involved in complex trait variation. To date, for many complex traits, tens to hundreds of loci have been identified that explain in total up to 20% of narrow sense heritability. The variation is spread over all chromosomes in proportion to their length, implying that there are many more variants with effects sizes too small to be detected with sample sizes employed to date. Empirical observations from the resemblance between relatives, from within-family segregation variance captured by markers and from population based association studies are all converging to a highly polygenic model of complex traits, with a surprisingly large proportion of additive genetic variation due to variants that are in linkage disequilibrium with common SNPs.

For body-mass-index (BMI), tens of loci have been identified through GWAS but in total they only explain a few percent of the phenotypic variation. There is emerging evidence that the narrow sense heritability of BMI has been over-estimated, and we will show results from empirical data consistent with a heritability of BMI of 40–50%.

O19 – Symposium Lecture
Genetic Determinants of Blood Pressure Response to Caffeine Drinking
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Background: The widely observed between-subject variability in cardiovascular and psychoactive responses to coffee may have a genetic basis.

Objective: We evaluated acute blood pressure (BP) and psychoactive responses to caffeine and explored whether they are influenced by candidate gene variants affecting caffeine metabolism (for cyto-
Nutritional B Vitamin Deficiency Alters the Expression of Key Proteins Regulating Vascular Smooth Muscle Cell Proliferation and Migration in the Aorta of Atherosclerotic Apolipoprotein E Null Mice

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Background: Low dietary B vitamin status is linked with an increased risk of human vascular diseases. This has been causally linked with hyperhomocysteinemia, which can induce oxidative stress, lipid oxidation and endoplasmic reticular stress in vitro and in vivo. However, there is evidence that folate status alone may modify cardiovascualr disease risk.

Objectives: We employed a proteomic and biochemical approach to determine whether nutritional folate deficiency or hyperhomocysteinemia, differentially alters metabolic processes directly linked with atherosclerosis in the aorta and heart of ApoE null mice.

Methods: Methods: We recruited 110 male healthy habitual moderate coffee drinkers refraining from coffee for the day preceding the study. Each subject underwent ambulatory BP monitoring at 6 min intervals for 2 h, as well as psychometric tests for alertness and reaction times. Each participant was administered, in a double-blind design, 40 mL of either a decaffeinated coffee preparation plus 3 mg/kg caffeine (“caf”) or the corresponding vehicle (“decaf”). The protocol was repeated 24 h later with the alternative preparation. Blood samples were collected for genetic, plasma caffeine and catecholamine evaluations.

Results: Compared with decaf, caf was associated with a significant increase in systolic and diastolic BP (SBP, DBP) (by 4±12 mm Hg for SBP and 3±10 mm Hg for DBP (mean±SD); P < 0.001 for both). Plasma caffeine and adrenaline increased after caf, but not decaf. Out of 11 gene polymorphisms analyzed, a relationship between ADOR2A TT variant and ΔSBP peak and between ADRA2B I variant and ΔSBP mean and peak was observed: these variants were associated with increased SBP responses to caf. There were also imbalances in the frequency of genetic polymorphisms in high responders vs low responders in psychometric tests.

Conclusions: Variability in the acute BP and psychoactive responses to coffee may be partly explained by genetic variation.

Results: Significant changes in the expression of both aorta and heart proteins were detected in response to multiple B vitamin deficiency (P < 0.05) and were strongly linked with lipoprotein concentrations measured directly in the aorta adventitia (P < 0.001). Pathway analysis revealed treatment effects in the heart related primarily to cellular and mitochondrial respiration (e.g. epoxide hydrolase, pyruvate kinase, succinate dehydrogenase). In contrast, the primary proteins and metabolic processes influenced by B vitamin status in the aorta were involved in cytoskeletal organisation, VSMC adhesion and invasiveness (e.g. fibrinogen, moesin, transgelin, vimentin).

Conclusions: Nutritional B vitamin deficiency induced striking changes in the expression of vascular proteins in atherosclerotic ApoE null mice. Deregulated expression of these proteins is associated with human atherosclerosis. Cellular pathways altered by B vitamin status included cytoskeletal organisation, cell differentiation and migration, oxidative stress and chronic inflammation. These findings provide new insight into the key molecular mechanisms through which B vitamin deficiency accelerates atherosclerosis.

Combination of Fish Oil and Curcumin Ameliorate Insulin Resistance by Increasing Serum Adiponectin and Expression of Fat Oxidation Marker PPARα and Decreasing Serum and Expression of TNFα in Obese Subjects and Diet-Induced Obese Mice

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Objectives: To clarify the effect of fish oil and curcumin on insulin resistance, body weight and their underlying mechanism in obese human subjects and to investigate their molecular mechanisms in obese mice

Methods: Twenty obese subjects were divided into 2 groups and treated either with fish oil and curcumin or placebo in cross-over design study. Both groups received dietary counselling to lose weight. Insulin resistance were measured with HOMA-IR and serum level of adiponectin, hsCRP and TNFα with ELISA. C57BL/6J obese and lean mice were used as animal model. The mice were then divided into 6 groups, group 1 lean control mice, group 2 obese control mice, group 3 obese mice with fish oil, group 4 obese mice with curcumin, group 5 obese mice with fish oil and curcumin and group 6 with metformin as positive control mice. IP-GTT and IP-ITT were measured for Insulin resistance and expression of adiponectin, PPARα and TNFα, in fat tissue and SRBEP1c in liver were measured by real time RT-PCR.

Results: In human obese subjects, compared to placebo groups TNFα was significantly dropped in treated group but HOMA-IR was insignificantly improved in treated group. Both groups lost weight in similar amount. On the other hand in mice, IP-GTT and IP-ITT
showed significant increase of blood glucose in all obese mice groups than those in lean mice. Compared to control obese mice, all treated obese mice had lower blood glucose except for fish oil group mice where surprisingly higher than obese control mice. Combination of fish oil and curcumin decreased blood glucose more than other groups. Expression of TNFα in fat tissue and SREBP1c in liver in all obese mice were significantly higher while expression of adiponectin in fat tissue and PPARα in liver were significantly lower compared to lean mice. Treatment with fish oil and curcumin decreased expression of TNFα, SREBP1c and increase expression of adiponectin and PPARα but not statistically significant.

**Conclusions:** Combination of fish oil and curcumin may be used as alternative adjunctive treatment for obesity-related insulin resistance.

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**Abstracts**

**O22**

**Interaction Between Genetic Variants in HNF1A and Erythrocyte Polyunsaturated Fatty Acids on the Risk of Type 2 Diabetes in Chinese Hans**

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**Objectives:** This study aims to assess the interaction between genetic variant in HNF1A and erythrocyte phospholipids polyunsaturated fatty acids (PUFAs) on the risk of type 2 diabetes (T2D) in Chinese Hans.

**Methods:** 622 patients with (T2D) and 293 healthy subjects were recruited in the present study. Erythrocyte phospholipids fatty acids were determined by gas chromatography. The rs7305618 in hepatocyte nuclear factor 1 alpha (HNF1A) gene was selected for genotyping by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF).

**Results:** The present study found that the major allele T can significantly increase the risk of T2D; the ORs for TC vs. CC and TT vs. CC were 1.53 (95% CI, 1.06 to 2.21) and 1.58 (95% CI, 1.04 to 2.38), respectively (P for trend <0.05). The rs7305618 exhibited significant interaction with erythrocyte phospholipids C18:2n6 and C20:4n6 on the risk of T2D (P for interaction <0.05). The risk allele T carriers (TT+CT) had a significant high risk of T2D than non-carriers (CC) only when subjects had a higher proportion of erythrocyte phospholipids C18:2n6 or C20:4n6, and the ORs were 2.59 (95% CI, 1.58 to 4.24) and 2.49 (95% CI, 1.47 to 4.24).

**Conclusions:** The present study suggested that proportions of C18:2n6 and C20:4n6 in erythrocyte phospholipids can modulate the effect of rs7305618 in HNF1A on the risk of T2D in Chinese Hans.

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**O23 – Keynote Lecture**

**Nutritional Modulation of Anti-Inflammatory and Pro-Resolving Signaling for Cancer Chemoprevention**

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The implication of inflammatory cell/tissue damage in pathophysiology of human cancer and some metabolic disorders is under intense investigation both at the research level and in clinical practice. Excessive and sustained inflammation causes DNA damage, genomic instability, epigenetic dysregulation and alteration of intracellular signaling, all of which are involved in neoplastic transformation. Numerous studies have identified a series of critical molecules/changes in the inflammatory signaling. In the case of inflammation-associated cancer, NF-κB and STAT3 are frequently overactivated. In contrast, Nrf2 counteracts NF-κB and STAT3, thereby exerting anti-inflammatory effects. The proper regulation of these redox-sensitive transcription factors mediating pro- or anti-inflammatory signaling hence provides important strategy for the chemoprevention of inflammation-associated carcinogenesis. Timely resolution of inflammation at the early stage is important in preventing further progression to chronic inflammation and related disorders including cancer. Resolution of inflammation is an active coordinated process regulated by distinct anti-inflammatory and pro-resolving endogenous lipid mediators, such as resolvins and protectins, which are derived from n-3 polyunsaturated fatty acids. The involvement of pro-inflammatory signaling in carcinogenesis has become more and more evident and well characterized, and a wide array of anti-inflammatory substances in our diet have been reported to exert chemopreventive effects. However, the anticarcinogenic potential of pro-resolving mediators remains still elusive. In searching for an efficacious way to prevent chronic inflammation-associated cancer, the pro-resolving as well as anti-inflammatory signal transduction pathways and their regulators merit further investigation.

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**O24 – Symposium Lecture**

**Discovering Connections Between Nutrigenomics and Preventive and Personalized Medicine with Insights from the Wisdom of Ayurveda**

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Preventive and personalized medicine continue to be one of the grand challenges of contemporary biomedical research. Significant progress has been made in uncovering the genetic basis of Mendelian
disorders. However, despite unprecedented technological advance-
ment, it has not been possible to explain the missing heritability in
common complex traits. Limited ability to identify the environmental
attributes and the clinical heterogeneity underlying most such disor-
ders have long been ascribed to be the major reasons for this failure.
A change from common disease common variant to common disease
rare variant hypothesis has also not yielded much success warranting
development of new paradigms.

Ayurvedomics is one such novel approach being fostered to
address a few limitations plaguing complex trait genetics. This stra-
tegy combines the use of contemporary genome analysis tools with the
subgrouping of individuals into three predominant prakriti groups
namely Vata, Pitta and Kapha based on the principles of Ayurveda, a
holistic Indian traditional system of medicine. According to this, the
body constitution termed as prakriti is determined by the physical,
psychological and physiological constitution of an individual.
Conditioning association studies on prior risk which is predictable by
these prakriti groups is hypothesized to reveal more variance and lead
to more predictive health. Understanding the relationship between the
nutritional recommendations, disease risk prediction and therapeutic
interventions for these subgroups of individuals, which is practised in
Ayurveda is another dimension of this novel approach. Supportive
results from our work carried out on Rheumatoid arthritis using this
approach and the larger implications of such an approach for nutri-
genomics and consequently preventive and personalized medicine will
be discussed.

O25

**Helicobacter pylori Induces Hypermethylation of CpG Islands in the Promoter Region of the 15-Hydroxyprostaglandin Dehydrogenase**

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**Objectives:** Helicobacter pylori (H. pylori) infection causes chronic gastritis and gastric cancer. H. pylori infection induces produc-
tion of prostaglandin E\(_2\) (PGE\(_2\)) implicated in pathogenesis of
gastric carcinogenesis. 15-Hydroxyprostaglandin dehydrogenase (15-
PGDH) is the key enzyme that catalyzes the conversion of PGE\(_2\) to
biologically inactive 15-keto-PGE\(_2\). Down-regulation of 15-PGDH
has been observed in gastric cancer. However, the molecular mecha-
nisms underlying regulation of 15-PGDH remain largely unknown in
stomach cancer. Therefore, this study was undertaken to elucidate
mechanisms responsible for down-regulation of 15-PGDH in rat gas-
tric mucosa (RGM)-1 cells and mouse gastric tissues infected with
H. pylori.

**Methods:** To investigate the level of methylation of 15-PGDH
promoter, methyl specific PCR was conducted in the cells infected
with H. pylori. The expression of 15-PGDH, DNMT (DNA methyl-
transferase)1, and Nrf2 was determined by Western blot analysis in
RGM-1 cells and C57BL/6 mice infected by H. pylori.

**Results:** H. pylori induced hypermethylation of 15-PGDH pro-
moter in RGM-1 cells. H. pylori induced expression of both mRNA
and protein of DNMT1. 5-Aza-2’-deoxycytidine (5-Aza-CdR), a spe-
cific inhibitor of DNA methylation, enhanced expression of 15-PGDH
in RGM-1 cells treated with H. pylori. H. pylori induced Nrf2 expres-
sion in the nuclear fraction and the whole lysate of RGM-1 cells.
H. pylori –induced DNMT1 expression was decreased by the ROS
scavenger N-acetylcysteine. In H. pylori-infected C57BL/6 mice,
15-PGDH expression was reduced while DNMT1 expression was
apparently increased in the stomach tissues. Curcumin, a major ingre-
dient present in turmeric, attenuated the down-regulation of 15-PGDH
expression in H. pylori-treated RGM-1 cells. Oral administration of
curcumin induced expression of 15-PGDH in mouse stomach in vivo.

**Conclusion:** H. pylori induced DNA methylation of CpG islands
in the 15-PGDH promoter through up-regulation of DNMT1 expres-
sion, which may contribute to the gastric carcinogenesis. Curcumin
induced 15-PGDH expression, which may account for its chemopre-
ventive effects on gastric carcinogenesis.

O26

**Dietary Factors in Crohn’s Disease: Tolerances and Intolerances to Vegetables in a New Zealand Caucasian Population**

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**Background:** Diet is known to play a major role in Crohn’s dis-
ease (CD). Most CD patients are aware of foods which have benefi-
cial or adverse effects on them. We investigated gene-diet association
on CD patients in a New Zealand Caucasian population.

**Methods:** A self-reported dietary questionnaire of tolerances and
intolerances to 224 food items in 12 groups, including 45 vegetable
items, was administered to 296 CD patients. These patients were also
genotyped using ImmunoChip. A total of 1044 SNPs in 374 genes
met the criteria for pathway analysis (p-value <0.01). Forty-three
pathways were selected for their prior relevance to inflammation.
Smoking status was adjusted for gene-diet association analysis.

**Results:** Sixteen items in 22 pathways were considered to be
beneficial while 15 items in 20 pathways were considered to have
adverse effects on CD patients. Two items (Kumara and roasted
potato) showed both beneficial and adverse effects depending on the
genotype may be beneficial to control symptoms in CD patients.
MST-312 Alters Telomere Dynamics, Gene Expression Profiles and Sensitises Human Breast Cancer Cells to PARP-1 Inhibition

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**Background:** Targeting telomerase is a potential cancer management strategy given that reactivation of telomerase allows for unlimited cellular replication in majority of the cancers. In mammalian cells, dysfunctional telomeres are recognised as double strand breaks (DSBs). However, status of DNA repair response pathways following telomerase inhibition is not well understood in human breast cancer cells.

**Objective:** We evaluated the effects of MST-312, a chemically modified derivative from tea catechin, Epigallocatechin gallate (EGCG) on telomerase and telomere homeostasis in MCF-7 and MDA-MB-231 breast cancer cells.

**Methods and Results:** MST-312 effectively reduced telomerase activity and induced growth arrest in MCF-7 and MDA-MB-231 breast cancer cells. DSBs and dysfunctional telomeres were detected using immunofluorescence and telomere-dysfunction-induced foci assays respectively in breast cancer cells. Anti-proliferative effects of MST-312 varies with cell type as MDA-MB-231 cells showed greater sensitivity to MST-312, with decrease in p21 and TRF2 protein levels compared to MCF-7 cells. Gene expression studies showed a decrease in expression of DNA repair genes, \( \text{RAD50} \) and \( \text{ATM} \), which are important in homologous recombination repair pathway in MDA-MB-231 cells. Western blot analysis confirmed reduction in expression of ATM protein in MDA-MB-231 cells.

**Conclusion:** These findings highlight the involvement of DNA repair proteins in regulating the cell sensitivity to telomerase inhibition induced growth arrest. In addition, inhibition of DNA repair protein, poly (ADP-ribose) polymerase in telomerase-inhibited cells, further reduced proliferation of breast cancer cells. Our findings suggest a potential for MST-312 to be used along with PARP inhibitors in the management of breast cancers.

Association and Interaction Effect between VEGF Receptor-2 (VEGFR-2) Gene Polymorphisms and Dietary Pattern on Blood Lipids in Malaysian Adults

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**Objectives:** This study aimed to determine the association and interaction effects between vascular endothelial growth factor receptor-2 (VEGFR-2) gene polymorphisms (rs1870377 and rs2071559) and dietary patterns on blood lipids in multi-ethnic Malaysian adults.

**Methods:** Dietary intakes of 509 (153 Malay, 179 Chinese, and 177 Indian) Malaysians were obtained from food frequency questionnaire for the construction of dietary patterns using factor analysis. Anthropometric measurements: body mass index and blood pressure; and biomarkers: glycated hemoglobin, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total cholesterol/HDL-C ratio were determined. Genotyping of rs1870377 and rs20171559 was performed by real-time PCR using Taqman probes.

**Results:** Two major dietary patterns were extracted from all subjects: ‘Vegetables, fruits, and soy diet’ (VFSD), and ‘Rice, egg, and fish diet’ (REFD). The allele frequency for rs1870377 (A allele; T allele) and rs2071559 (C allele; T allele) in Malays were (0.47;0.53 and 0.41;0.59), Chinese (0.52;0.48 and 0.38;0.62) and Indians (0.15;0.85 and 0.57;0.43) respectively. Significant associations were obtained between ethnic groups and blood lipids: TC (p = 0.004); LDL-C (p < 0.001); and HDL-C (p < 0.001). In Malays, significant associations were obtained between dietary pattern (REFD) and TG (p = 0.002) and HDL-C (p = 0.004); and VEGFR-2 gene polymorphism (rs2071559) with TG (p = 0.048). In Chinese, only significant associations were obtained between dietary pattern (REFD) and TG (p = 0.002) and HDL-C (p = 0.004); and VEGFR-2 gene polymorphism (rs2071559) with TG (p = 0.048). The interaction effects of VEGFR-2 gene polymorphisms and dietary patterns were significant in Malays between rs2071559 and REFD on TG (p = 0.005); and in Chinese between VFSD and rs1870377 on LDL-C (p = 0.028) after adjusting for potential confounders. There were no significant genetic or dietary associations on blood lipids in Indians (p > 0.05).

**Conclusions:** The significant associations and gene-diet interaction effects between VEGFR-2 gene polymorphisms and dietary patterns on blood lipids may pose hyperlipidemia and hypercholesterolemia risks in Malay and Chinese Malaysians.
O29

Kiwifruit Improves the TG/HDL-Cholesterol Ratio in Individuals with the CETP TAQ1B B1/B1 Genotype

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Background: Genetic variation may contribute to inter-individual variability in the effects of fruit interventions on HDL cholesterol (HDL-C).

Objectives: To assess the effects of four targeted HDL-C-related polymorphisms (CETP Taq1B; APOA1 –75G/A; LIPC –514C→T; LIPG 124582) on the plasma lipid response to a kiwifruit intervention in 87 hypercholesterolaemic men.

Methods: After a 4-week healthy diet run-in, subjects were randomized to one of two 4-week dietary intervention sequences of two green kiwifruit as part of a healthy diet would be particularly beneficial in hypercholesterolaemic men with this genotype.

Results: Genotype data was available for 82 subjects. Based on initial multivariate analysis of variance screening (comparing major allele homozygotes versus minor allele carriers), only the initial multivariate analysis of variance screening (comparing major allele homozygotes versus minor allele carriers) was taken at baseline-1, after run-in (baseline-2) and end of crossed-over treatment periods. This retrospective analysis was a secondary objective of the study.

Conclusions: Although moderate, the significant improvement in TG/HDL-C ratio of B1/B1 homozygotes, a group at higher risk of atherogenic lipoprotein phenotype, suggests that regular inclusion of green kiwifruit as part of a healthy diet would be particularly beneficial in hypercholesterolaemic men with this genotype.

The trial was registered with the Australian-New Zealand Clinical Trials Registry (no. ACTRN12610000213044).

O30

Diverse Effects of Resistant Starch and Red Meat on Proliferation and O6Methyl-2-Deoxyguanosine Adduct Formation in the Distal Colon of Msh2 Deficient Mice: Consequences for Colorectal Carcinogenesis

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Background: Red meat can increase pro-mutagenic lesions in the colon of humans and rodents, whereas resistant starch (RS) can reduce pro-mutagenic lesions and colonic tumours in rodent models. Mice lacking Msh2 develop more CRC when exposed to alkylating carcinogens.

Objective: We determined if red meat and RS could modify pro-mutagenic adducts and proliferation in Msh2 deficient mice and whether this influenced colonic carcinogenesis.

Methods: 100 Msh2+/- and 60 Msh2+/+ mice were assigned to 4 diets (Control, RS, Red Meat and Red Meat+ RS) for 6 months. Colonic aberrant crypt foci (ACF), colon tumours and lymphoma were examined. 12 mice from each dietary treatment group were measured for O’Methyl-2-deoxyguanosine (O’MeG) and Ki-67 in paraffin embedded distal colons.

Results: Msh2+/- mice consuming red meat survived for significantly longer than mice on control diet (p < 0.01). Surprisingly, Msh2+/- mice fed red meat had significantly less O’MeG adducts compared to wild type mice (p < 0.05). O’MeG adducts in wild type mice were also significantly reduced by RS treatment (p < 0.05). Proliferation was higher in Msh2+/- mice than in wild type mice receiving either control or red meat (p < 0.05). RS restored proliferation in Msh2+/- mice to wild type levels. No colon tumours were observed. There was no difference between diet treatment and genotype on ACF or lymphoma.

Conclusions: The unexpected finding was red meat reduced formation of pro-mutagenic DNA lesions in distal colon and increased survival time in Msh2+/- mice. Development of lymphomas in the Msh2 mice limited duration of the dietary treatments to 6 months and as such no colon tumours were detected in the current model. However, the detected changes in adduct formation and proliferation did not influence colonic tumourigenesis. Repair of O’MeG by methyl-guanine-methyl-transferase (MGMT) could be different in Msh2+/- mice and further investigations are justified.
Omega-3 Polyunsaturated Fatty Acids Selectively Inhibit Growth in Squamous Cell Carcinoma Oral and Epidermal Keratinocytes by Differentially Activating ERK1/2

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Objectives: The aim of the study was to investigate the potential of omega-3 PUFAs as selective chemopreventive and therapeutic agents against oral and epidermal SCCs and their mechanism of action.

Methods: The effect of omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on oral and epidermal malignant SCC, pre-malignant and normal keratinocytes was examined by MTT cell viability assay. Apoptosis was tested by the flow cytometric annexin V apoptosis assay and by caspases cleavage with western blotting while cell proliferation was examined by the 3H-thymidine assay. Reactive oxygen species (ROS) production was investigated by the flow cytometric DCF assay and DNA damage by immunocytochemistry. The signaling pathways involved were examined by western blot followed by blockage of specific targets with inhibitors.

Results: Low doses of the omega-3 PUFAs, and EPA in particular, inhibited the growth of premalignant and malignant keratinocytes more than the growth of normal counterparts by a combination of cell cycle arrest and apoptosis. The growth inhibition of the oral SCC lines, but not normal keratinocytes, by both omega-3 PUFAs was associated with epidermal growth factor receptor (EGFR) autophosphorylation, a sustained phosphorylation of ERK1/2 and its downstream target p90RSK. Inhibition of EGFR with either the EGFR kinase inhibitor AG1478 or an EGFR-blocking antibody inhibited ERK1/2 phosphorylation, and the blocking antibody partially antagonized growth inhibition by EPA. DHA generated more ROS and activated more JNK than EPA, potentially explaining its increased toxicity to normal keratinocytes.

Conclusions: DHA and EPA, in particular, display a marked anti-tumour effect against SCC keratinocytes at concentrations that do not eliminate normal cells, by a sustained signalling imbalance amplifying the EGFR/ERK/p90RSK pathway in neoplastic keratinocytes. There is a significant potential in their use as future therapeutic and prophylactic tools against epidermal and head and neck cancer.

Nutrigenomics Tools Workshop

A Network Science View of Nutrigenomics and Nutrigenetics

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The interpretation of an environmental signal into an appropriate gene expression response is partly mediated through a cohort of ligand-activated transcription factors called Nuclear Hormone Receptors (NHR). Example ligands for NHR include fatty acids and hormones. The NHR PPARG (NR1C3) binds free fatty acids, homodimerises with RXR and binds DNA at a particular consensus sequence. This culminates in a transcriptional response influencing the behaviour of ‘fat burning’ peroxisomes and mitochondria. A key feature of NHR is that their messenger RNA expression level tends to be relatively stable, whether ligand-activated or not. What changes is their behaviour at the protein level – during activation they trans-locate to the nucleus and bind DNA even though their mRNA expression level may not be upregulated. This phenomenon means they are very challenging to identify by the conventional approach of ranking transcripts on differential expression (DE). Here we show how a differential network approach (called differential wiring or Regulatory Impact Factors) can be exploited to prioritise those regulatory molecules whose behaviour has changed, irrespective of DE – thereby giving a more accurate picture of the responsiveness of the genome to nutritional and other environmental stimuli. Secondly, we show how genetic variant (SNP) data can also be used in an analogous process to prioritise gene variants in NHR and other molecules who associate with a nutritionally-responsive phenotype. This is achieved through monitoring their change in location across two co-association networks. Overall, we show how network science tools developed by our group can shed fresh light on our understanding of NHR biology in both genomic (mRNA) and genetic (SNP) data.
for expression in different cell types. In humans, there is now clear evidence of reproducible and site-specific changes in DNA methylation of children born to mothers who smoked during pregnancy.

Among epigenetic modifications, methylation of DNA at cytosines at CpG dinucleotides is the most readily studied. The emergence of genome-wide approaches for simultaneously measuring methylation at large number of CpG sites or regions is transforming approaches to understanding the impact of nutritional and environmental challenges on the epigenome and their potential role in modifying phenotype. Whole genome bisulphite sequencing is able to provide quantitative analysis of all CpG sites in the genome, but is prohibitively expensive for studies involving large sample numbers. Hence, most studies use platforms that assess methylation at a subset of sites across the genome. The most widely used technology at present is the Illumina Human Methylation BeadChip450 array that interrogates about 4,80,000 of the 29 million CpG sites in the genome. Sequence-capture methods that provide for high throughput bisulphite sequencing of selected regions of the genome (Roche/Nimblegen and Agilent, targeting 3 to 5 million CpGs) are also emerging. I will discuss the advantages and disadvantages of the different methods, analytical approaches and issues to be aware of, such as polymorphisms and cross-hybridising probes.

Objective: Nutrigenomics New Zealand (NuNZ; www.nutrigenomics.org.nz) has invested considerable time and effort researching the role of nutrient-gene interactions in the development, and potential prevention or treatment, of human inflammatory bowel disease (IBD). This research has utilised a number of ‘omics’ techniques, of which proteomics and metabolomics will be the focus for this talk.

Methods: Models of intestinal inflammation, including Il10 and Mdr1a gene-deficient mice, have been used to investigate the mechanisms underlying human IBD and to test foods or food components for potential beneficial effects. A proteomics approach using two-dimensional gel electrophoresis combined with LC-MS analysis of peptides for differential in-gel expression analysis, and metabolomics analyses using both GC- and LC-MS, have been applied in combination with microarrays (transcriptome) and next-generation sequencing (microbiome) for a comprehensive assessment of tissue, plasma and urine samples derived from these models.

Results: Across several independent studies, we have identified key proteins and metabolites which are involved in chronic inflammation. More importantly, we have also identified food compounds such as polyphenols (green tea, curcumin) or polyunsaturated fatty acids (EPA), or whole foods such as salmon or broccoli, that can reduce inflammation by regulating the activity of these proteins and metabolites. The proteins identified are linked to cellular stress and energy metabolism pathways, and metabolites include xanthurenic acid, 5-aminovaleric acid, hexanoylglycine, and cytosine.

Conclusions: There is no doubt that the various ‘omics’ techniques such as proteomics and metabolomics have deepened our understanding of the mechanisms underlying the inflammation associated with human IBD, and how nutrient-gene interactions may act to influence this inflammation. However, challenges remain in dealing with the enormous quantity of data generated by these techniques, and in harnessing this data to turn it into information that is both relevant and useful to improve the outcome for people living with IBD.

The health of the individual and the population in general results from the interactions between genetic and environmental factors. Diet (Nutrition) is an environmental factor of major importance, both in the prevention of chronic diseases, in normal development, and in the maintenance of homeostasis. Studies on genetic variants and their response to diet in health and disease (Nutrigenetics) as well as studies on the role of nutrients in gene expression (Nutrigenomics) will lead the way in the development of personalized nutrition, in the definition of a healthy diet, and in the development of Novel Foods. To continue in its role as an integrator of genomic and environmental processes, Nutritional science is adjusting its focus to include the microstructure of the genome, the metabolome, the proteome, the epigenome, metagenome, etc. Ultimately, we will be able to understand nutritional metabolism at levels of discrimination sufficient to permit individual dietary prescription. In contemplating how genetics and Nutrigenetics/Nutrigenomics will look in the future, we can foresee that Genetics will not remain the exclusive prerogative of Departments of Genetics or Regional Genetic Centers. Instead, every physician and other members of the “healthcare team” will need to use genetic knowledge and combine it with appropriate dietary regimen, type and amount of physical activity, and when needed drugs. Nutritional modulation of epigenetic processes adds a further layer of complexity to gene-nutrient interactions and should be considered in the definition of strategies for health promotion and disease prevention. Because epigenetic marks are potentially reversible and are implicated in the pathogenesis of diverse non-communicable diseases representing major public health problems in both developed and developing countries, the epigenome becomes an attractive target for nutritional intervention. Public health and regulatory processes will need to be established to define when genomic discoveries such as gene/nutrient disease associations are ready to be evaluated as potential tools to improve health screening and recommended dietary levels.
Objective: Iron intake has been shown to be associated with DNA damage. It has also been proven that DNA damage in pregnant women associated with the incidence of preeclampsia, intrauterine growth restriction (IUGR), and other adverse pregnancy outcome. Since DNA damage can occur in the fourth week of pregnancy, then nutrition intervention should be started on preconception period. This study aims to assess the association between mean corpuscle volume (MCV) – iron status parameters in red blood cells-, with oxidative DNA damage in preconception women. This study is part of an umbrella research on the effect of multi micronutrient supplement in reducing DNA damage in preconception women in Indonesia, from January to May 2013. Sixty-four preconception women that meet the criteria enrolled in the study. Iron parameters were measured using the SLS-Hemoglobin method, while oxidative DNA damage (8-hydroxydeoxiguanosine) 8-OHdG levels were measured using ELISA. Data analyzed using Spearman Correlation.

Methods: The study design was a cross sectional study, conducted in Ujung Tanah and Biringkanaya sub-districts of Makassar, Indonesia, from January to May 2013. Sixty-four preconception women that meet the criteria enrolled in the study. Iron parameters were measured using the SLS-Hemoglobin method, while oxidative DNA damage (8-hydroxydeoxiguanosine) 8-OHdG levels were measured using ELISA. Data analyzed using Spearman Correlation.

Results: Eleven samples (17%) of the total sample have mean corpuscle volume (MCV) below normal with mean score 83.42 and SD ±6.2. Mean score of 8OHdG was 36.08 and SD ±2.17. There was negative association between MCV and 8-hydroxydeoxiguanosine (p = 0.033).

Conclusion: Negative association between MCV and 8-hydroxydeoxiguanosine indicate that the higher of MCV the lower of DNA damage.

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Folate Nutrigenomics/Nutrigenetics and Pre-Eclampsia: A Systematic Review
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Objectives: The aim of this review is to perform a systematic literature review to identify potential knowledge gaps in relation to the folate nutrigenomic/nutrigenetic profile in women at risk of Pre-eclampsia (PE). The articles were also reviewed for the possible role of folate in either the aetiology or prevention of PE.

Methods: A systematic literature search strategy was implemented designed to identify relevant articles from electronic databases including the terms Nutrigenetics AND Pre-eclampsia, Nutrigenomics AND Pre-eclampsia, Folic acid supplementation AND Pre-eclampsia AND genome stability AND DNA methylation. The criteria for study inclusion were as follows:
(i) Studies/ reviews that evaluated effect of folic acid supplementation and/or folate status in women with PE; (ii) The role of folate status/supplementation studied in context of genotype differences or genome stability events or global/gene specific methylation patterns studied in tissues of women at risk of PE; (iii) Only full-text English language articles.

The articles were assigned a level of evidence, according to Australian National health and Medical Research Centre’s criteria.

Results: Of the 1123 articles retrieved, only 50 articles were finally selected according to predefined selection criteria and grouped into FA supplementation and PE (n = 12), genome stability and PE (n = 7), Folate DNA methylation and PE (n = 22), Folate nutrigenetics and PE (n = 9). The diverse subject group and different type of variables studied across the articles selected prohibited statistical assessment of heterogeneity and subsequent meta-analysis. The current review found evidence that women at increased risk of PE may also be hypersensitive to genome instability events and to alterations in placental DNA methylation patterns. The review also found inconsistent data based on highly heterogeneous non coherent studies on the possible role for folic acid supplementation in the prevention of PE.

Conclusions: The effect of folate Nutrigenomic/Nutrigenetic associations in relation to women at risk of PE requires further exploration and may prove to have an important role in terms of future diagnostics and preventative measures.

Juçara (Euterpe edulis Mart.) Supplementation During Pregnancy and Lactation Offspring Alters Gene and Proteic Expression of Inflammatory Markers in the Colon of 21-D-Old Pups
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Objectives: To evaluated the effect of pulp Juçara 0.5% diet supplementation about the trans fat acid (TFA) intake during pregnancy and lactation on the inflammatory status of 21-d-old offspring.

Methods: On the first day of pregnancy rats were divided into three groups: control diet (C), diet enriched with hydrogenated vegetable fat, rich in TFA (T), and T supplemented with 0.5% Juçara freeze dried (TJ). Dam’s diet were maintained during pregnancy and lactation. At 21 th, pups were decapitated. Glucose, triacylglycerols, cholesterol serum concentrations were measured by an enzymatic colorimetric method (Commercial kits Labtest, Brazil). TNF-α, IL-6 and IL-10 proteins was measured by ELISA (DuoSet, R&D Systems). Gene expression levels of TNF-α receptor and Tool like receptor (TLR4) in colon were determined by real-time PCR. Results are expressed in arbitrary units. Statistical analysis was performed with ANOVA, Bonferroni t-test or Kruskal-Wallis. p < 0.05.

Results: Juçara (0.5%) supplementation during pregnancy and lactation reduced serum Glucose (*C = 108.54; *T = 113.8; *TJ = 98.5 mg/dL), triacylglycerols (*C = 167.7 μg/dL; *T = 188.0; *TJ = 134.2 mg/dL) and cholesterol (*C = 115.4; *T = 133.8; *TJ = 107.8 mg/dL) (**p < 0.05). Juçara (0.5%) supplementation reduced mRNA levels of TLR4 (*C = 0.802±0.175; &T = 2.267±0.507; *TJ = 0.760±0.224) and TNF-αR (*C = 0.727±0.061; &T = 1.423±0.194; *TJ = 0.825±0.146) (**p < 0.05), accompanied by an reduced in the IL-6 (*C = 25.1±1.4; &T = 34.2±3.1; *TJ = 18.9±1.5 pg/μg) and TNF-α (*C = 13.9±0.7; &T = 18.9±1.5; *TJ = 12.1±1.0 pg/μg) in pup’s colon compared to T group (**p < 0.05). Diet enriched with TFA during pregnancy and lactation decreased IL-10 (*C = 57.7±4.0; &T = 36.5±5.6; *TJ = 33.1±3.1 pg/μg) (**p < 0.05).

Conclusions: Diet enriched with TFA increase the pro-inflammatory status and Juçara 0.5% diet supplementation during pregnancy and lactation decrease the pro-inflammatory status in 21-d-old pups colon, it is could contributes with reduced chronic disease development.
**P005**

**Effects of Maternal Obesity and Exercise During Pregnancy on Expression of Cardiac Taste Receptors in Weanling Rat Offspring**

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**Objectives:** Growing experimental and clinical evidence indicates that maternal obesity leads to adverse outcomes for offspring, including cardiac pathologies. Changes in taste preference of offspring from mothers consuming a high fat diet (HFD) have also been reported. Taste receptors were recently described in the mouse heart, and shown to be regulated during development. In this study it was postulated that maternal obesity would modulate the expression of taste receptors in the heart, along with other cardiac genes. Effects of maternal exercise were also investigated.

**Methods:** Female Sprague-Dawley rats were fed chow (C) or HFD (F) and half of each were provided with a running wheel and underwent voluntary exercise (CE or FE) from 10 days prior to mating, whilst the others remained sedentary (CS or FS). Two pups from each mother were sacrificed at postnatal day 19 and cardiac taste receptors were measured by RT-PCR.

**Results:** Both lean and obese dams undertook similar amounts of exercise (8.1±2.4 vs 5.1±1.5 km total). Maternal obesity increased offspring body weight, adiposity, heart ventricle mass (all P < 0.05) and leptin concentration (P < 0.01), with no effect of exercise. Heart mass remained heavier after correction for body weight. Cardiac ventricle mRNA expression of bitter type 2 taste receptors and beta adrenergic receptor were decreased in response to maternal HFD with no effect of exercise. Obesity and exercise had no impact on sweet receptors. Cardiac FTO mRNA expression was also down-regulated by maternal HFD with an up-regulation in the offspring of CE mothers.

**Conclusions:** Maternal obesity selectively affected the expression of bitter taste receptors and other genes in the cardiac ventricle of weanling rats. Further work is required to determine the function of these receptors, and whether these changes are implicated in the development of cardiac pathologies associated with maternal obesity.

**P006**

**Impact of Variants in Folate and Methionine Cycle Genes on Homocysteine Levels and DNA Methylation and Their Association with Schizophrenia in South Indian Population**

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**Objectives:** To investigate the association of polymorphisms in folate and Methionine cycle genes with schizophrenia in a south Indian population. To analyse the levels of homocysteine in plasma and Global DNA methylation in patients and controls. To study the impact of variants in Folate and Methionine cycle genes on homocysteine levels and DNA methylation.

**Methods:** DNA was isolated from 300 patients and 300 healthy controls after obtaining informed consent. Only patients suffering from schizophrenia diagnosed by DSM-IV/ICD 10 have been enrolled for this study. Symptom severity was rated using BPRS-E and PANNS. Age, sex and ethnicity matched controls were recruited for the study. We selected 16 SNPs from Methionine cycle genes that include MTHFR, MTR, MTRR, CBS, SHMT and DHFR genes based on their functional relevance. Samples were genotyped using Sequencing and PCR-RFLP. Homocysteine levels in plasma and Global DNA methylation levels in peripheral blood lymphocytes were analysed using ELISA based techniques.

**Results:** Mean Homocysteine levels in patients were found to be significantly higher compared to controls. Homocysteine levels were also found to be higher in patients with MTHFR risk genotypes (677CT and 1298AC and 1298CC). Interestingly these variants were not found to be associated with Schizophrenia. In another Methionine cycle gene, the MTRR, AG genotype of rs1532268 (p = 0.006) was found to be associated with Schizophrenia.

**Conclusions:** High levels of Homocysteine coupled with altered methylation may be considered as a biomarker in schizophrenia. Screening for associated variants in Folate and Methionine cycle genes might help in designing treatment strategies involving nutritional intervention.
**P007**

The Effect of Furan Fatty Acid Against DNA Damage and Cytotoxicity in Astrocytoma Cell Lines Challenged with Oxidative Stress

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**Objectives:** Fatty acids from fish have been associated with improved brain function. These benefits may partly be attributed to the presence of furan fatty acids (FFA) in fish oil (1%) which exhibit potent antioxidant properties and may potentially protect brain astrocytes against oxidative damage. Understanding their effects in astrocytes is important as these cells are responsible for maintaining healthy neurons and their decline with ageing is a possible cause of dementia. The aim of this study was to investigate the cytotoxic and genotoxic effects of FFA on *in vitro* astrocytoma cell cultures, U87MG (APOE ε3/ε3) and U118MG (APOE ε2/ε4) with and without an acute hydrogen peroxide (H2O2, 100 μM) challenge.

**Methods:** Our primary outcome measure, the Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay, a comprehensive and well-validated assay, allowed us to measure DNA damage biomarkers such as micronuclei (chromosome loss and/or breakage), nucleoplasmic bridges (DNA misrepair and/or telomere end fusions) and nuclear buds (gene amplification) as well as rates of cell proliferation and cytotoxicity (i.e. apoptosis and necrosis).

**Results:** The cell lines firstly differed in their sensitivity to H2O2 challenge. U118MG was found to be more sensitive to the cytostatic, cytotoxic (i.e. apoptosis) and DNA damaging effects (micronuclei-MNi, nucleoplasmic bridges-NPB, and nuclear buds-NBUDs) of H2O2 (p < 0.01 – p < 0.001) when compared to U87MG. The effects of FFA also differed between the cell lines, with significant effects observed in decreased baseline cytostasis (p = 0.0022) in the U87MG cell line, while increasing cytostasis (p = 0.0144) in the U118MG cell line. However, no significant interaction with H2O2 and FFA were observed for U118MG, while significant interactions were found for a reduction in cellular proliferation (p = 0.0161) and necrotic cell frequency (p < 0.0001) in U87MG cells.

**Conclusions:** Overall, the effects of FFA varied between cell lines with regards to cytostasis and exerted minimal effects on DNA damage biomarkers.

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**P008**

Chromosome 17 and 21 Aneuploidy in Buccal Cells and their Association with Plasma Folate, B12 and Homocysteine in Alzheimer’s Disease

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**Objectives:** Alzheimer’s disease is a premature ageing syndrome characterised by cognitive impairment arising from neuropathological changes occurring within specific areas of the brain. The aim was to use fluorescently labelled DNA probes, to determine the incidence of aneuploidy of chromosome 17 and 21 in buccal cells of Alzheimer’s patients compared to age and gender matched controls. Buccal cells were collected for this study because they can be collected non-invasively and originate from the neuroectoderm from which brain tissue is derived. Buccal cells may therefore exhibit genetic defects common to brain tissue acquired during the early stages of development.

**Methods:** Buccal cells were collected using a standardised protocol from newly diagnosed Alzheimer patients prior to treatment and age and gender matched healthy controls. Centromeric chromosome 17 specific DNA probes were generated by PCR labelling with centromere specific primers. Chromosome 21 aneuploidy was investigated using a directly labelled commercially bought fluorescent probe labelled with spectrum orange. Both probes were validated by hybridising to human metaphases. Fluorescent in situ hybridisation was performed to determine the rates of aneuploidy by fluorescent spot counting. Plasma folate, B12 and homocysteine were quantitated using the ARCHITECT folate, B12 assays and the AxSYM homocysteine assay.

**Results:** A significant 1.5 fold increase in trisomy 21 (p < 0.001) and a 1.2 fold increase in trisomy 17 (p < 0.001) was found in buccal cells of Alzheimer’s patients compared to the control group. Homocysteine was found to be significantly increased (p = 0.0003) in the AD cohort. A positive correlation was shown between homocysteine and monosomy of chromosome 17 (p = 0.03)

**Conclusions:** These results are suggestive that the aneuploidy events investigated which are increased beyond the incidence in normal ageing may be influenced by genetic factors that may predispose to AD, and may also be influenced by dietary factors leading to altered homocysteine status.
P009

Nutritional and DNA Damage Markers in Autism

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Objectives: Abnormal plasma levels of metabolites in the folate-dependent methionine and glutathione metabolism pathways have been reported in children with autistic disorder compared to age-matched controls. Cytogenetic alterations in peripheral blood lymphocytes and/or buccal cells have been reported in a range of neurologic conditions. It was hypothesised that children with autistic disorder may have increased levels of DNA damage compared to their non-autistic siblings or community controls.

Method: DNA damage bio-markers as measured by the cytokinesis blocked micronucleus assay and plasma levels of B vitamins and homocysteine were measured in 35 children with autistic disorder may have increased levels of DNA damage compared to their non-autistic siblings or community controls.

Results: There was no significant difference in DNA damage bio-markers between children with autism, their non-autistic siblings and community controls suggesting that autistic disorder is not associated with a genetic susceptibility towards DNA damage induced by endogenous toxins and/or poor nutrition or lifestyle.

Conclusions: Unlike some neurologic disorders, there appears to be no link between DNA damage and autistic disorder. The role of riboflavin in the aetiology of autism requires further investigation.

P010

Coordinated Expression of Selected Cytokine and Zinc Transporter Genes in Women with Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) is associated with a state of low-grade systemic inflammation and altered zinc metabolism. Intracellular zinc homeostasis is largely regulated by metallothionein (MT), an intracellular zinc buffering system, and 2 classes of zinc transporters: ZnT and ZIP. ZnTs decrease cytoplasmic zinc while ZIPs increase cytoplasmic zinc concentration.

Objective: To investigate the effects of zinc supplementation on the gene expressions of cytokines and zinc transporters, and their inter-relationships in post-menopausal women with T2DM.

Methods: Post-menopausal women with T2DM (n = 48) were randomised into zinc supplemented (40 mg elemental zinc/d) or placebo groups for 12 weeks. At weeks 0 and 12, peripheral blood mononuclear cells were isolated and total RNA extracted. Expression of zinc transporter, MT and cytokine mRNA were quantified using real time-PCR.

Results: Gene expression of TNF-α was up-regulated by 27% with zinc treatment (p < 0.05). No significant differences were observed in zinc transporter expressions after zinc treatment. Multivariate analysis of variance between IL-6 or TNF-α and zinc transporter expressions did not show any significant relationships. IL-1β expression predicted expression of all zinc transporters and MT measured at week 0, with significant univariate correlations observed with ZIP1 and ZIP7 (p < 0.01). Overall multivariate relationships were maintained between IL-1β and zinc-related transcripts at week 12 with significant univariate relationship between IL-1β and ZnT7 (p < 0.01). A positive correlation between fold change of IL-1β and ZnT7 was observed in the zinc-treated group (p < 0.05) which was not found to be significant in the placebo group.

Conclusion: Zinc supplementation led to up-regulation of selected cytokines in T2DM. Change in IL-1β expression secondary to zinc supplementation was correlated with change in expression of ZnT7, one of the major zinc transporters responsible for zinc efflux into secretory pathway.

P011

Extracts of Chilean Native Fruits Inhibit Inflammation, Oxidative Stress and Insulin-Resistance of Adipocytes Induced by Macrophage Conditioned Media Treatment

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Objectives: To evaluate whether extracts of Chilean native fruits have the ability to inhibit the presence of an inflammatory and pro-oxidant response, and of insulin-resistance in adipocytes treated with conditioned media (CM) from macrophages.

Methods: Ripe fruits of Aristotelia chilensis (ACh), Berberis microphylla (BM) and Vaccinium corymbosum (VC – as control) were dried, pulverized, extracted in methanol:water, and rota-evaporated. 100 μM [polyphenols] of each extract were utilized. 3T3-L1 mouse adipocytes were treated with CM from previously LPS-activated macrophages in presence or absence of extracts for 24 h/96 h. Gene expression and secretion of inflammatory markers, antioxidant content, and glucose uptake were determined.

Results: CM induced higher and lower gene expression and secretion of MCP-1 and adiponectin, respectively, in adipocytes after
Hypoglycemic and Anti-Inflammatory Effect of Actinidia arguta Shoot in Mice Fed a High-Fat, High-Sucrose Diet

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**Objectives:** There is causative relationship between inflammation and insulin resistance. The purpose of this study was to investigate the effect of the shoot of *Actinidia arguta* on insulin resistance and inflammation in mice with diet-induced insulin resistance.

**Methods:** Five-week-old male C57BL/6J mice were fed a basal or a high-fat, high-sucrose (HFHS) diet with or without a 70% ethanol extract of *A. arguta* shoot at a 1% level of the diet for 12 weeks after 1 week of adaptation. After sacrifice, serum glucose, insulin, and adiponectin were measured and homeostasis model assessment for insulin resistance (HOMA-IR) was determined. Western blotting was performed to measure protein expression levels of monocyte chemotactic protein (MCP)-1 and interleukin (IL)-6 in the liver.

**Results:** Feeding with a HFHS diet increased serum glucose, insulin, HOMA-IR values, and expression of MCP-1 and IL-6 compared with the control group. Serum glucose and insulin, as well as HOMA-IR, were significantly lower in the *A. arguta* group than in the HFHS group. Serum adiponectin levels of the three groups were not significantly different. *A. arguta* shoot extract significantly decreased expression of MCP-1 and IL-6 compared with the HFHS group.

**Conclusions:** These findings suggest that *A. arguta* shoot may be useful in improvement of insulin resistance and hyperglycemia, partly by reducing expression of pro-inflammatory cytokines. (This research was supported by the Globalization of Korean Foods R&D program, funded by the Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea.)
enzyme is known to protect against lipoproteins oxidation and against the development of metabolic syndrome (MetS) manifestations. The aim of the current research was to study the potential relationships between the PON1 ARE activity and anthropometrics, biochemical markers, dietary antioxidants and the PON1 gene methylation levels, in volunteers with MetS symptoms after following a hypocaloric diet.

Methods: Adults with MetS features (n = 47, 47±10 y.o; BMI 36.2±3.8 kg/m²; 46.8% female), who followed a six-month energy-restricted dietary trial, were enrolled in the intervention. PON1 transcriptional regulatory region methylation was analysed using validated protocols (microarray) at the beginning of the study. Anthropometric, biochemical, enzymatic and dietary data were also assessed before and after the diet implementation.

Results: Participating subjects decreased body weight, BMI, total fat mass, diastolic blood pressure, mean blood pressure and triglycerides accordingly to the ARE activity reduction. Methylation levels of PON1 gene were positively associated with ARE activity at baseline. Noteworthy, dietary tocopherols, lycopene and vitamin C intakes, were positively correlated with ARE activity at the end of the study and showed a negative association with the methylation of some CpG sites of the PON1 gene at baseline.

Conclusions: Volunteers with MetS symptoms following a hypocaloric diet decreased ARE activity in parallel with MetS-related markers. Interestingly, dietary antioxidants might have a role in the enhancement of the ARE activity by lowering the PON1 gene methylation levels.

P015
Prediction of Obesity Through a Genetic Predisposition Score
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Objectives: The aim of the present study was to assess the value of a genetic predisposition score (GPS) on obesity prediction. The GPS is based on 18 independent genetic variants previously associated with obesity, lipid levels, glucose metabolism and blood pressure.

Methods: A total of 640 users of a Nutrigenetic Service were examined for anthropometrics, body composition, food habits and physical activity. Oral epithelial cells were collected for the identification of 18 SNPs, located in FTO, MC4R, MTHFR, PPARA, PPARG, APOA5, APOE, LIPC, PLIN1, NOS3, GCKR, LPL, CELSR2, CETP, LIPG, GNB3 and MTNR1B genes using DNA Zip Coded Beads. Genotypes were coded 0, 1 or 2 according to the number of risk alleles and the GPS was calculated by adding risk alleles with such criterion.

Results: About 23% of the users were male (n = 145) and 77% were female (n = 495), with an average age of 50.0±13.6 y.o., being 35.9% overweight and 40.3% obese. After adjusting for gender, age, physical activity and energy intake, the GPS demonstrated that individuals carrying ≥10 risk alleles had significantly higher obesity risk (OR: 1.53, 95%CI: 1.08–2.16) compared with the reference group (≤9 risk alleles). Indeed, the users with high genetic predisposition to obesity (≥10 risk alleles) had in average 0.86 kg/m² of BMI, 1.77% of body fat and 2.08 cm of waist circumference more than the users with low genetic predisposition to obesity (≤9 risk alleles). The discriminative accuracy of the GPS, evaluated by the area under the curve (AUC), was 0.73 (95%CI 0.08–0.77).

Conclusions: The GPS confirmed that the individuals with high genetic predisposition to obesity showed greater values of adiposity than the individuals with low predisposition. Furthermore, the GPS demonstrated a good discriminatory value for the obese phenotype.

P016
 Peroxisome Proliferator Activated Receptor γ Co-Activator 1α (PGC-1α) Expression in Skeletal Muscle of Diabetic Rats Increased after Diet with Lesser Yam Starch (Dioscorea esculenta) and Butyrogenic Bacteria Eubacterium rectal
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Background: Lesser yam (Dioscorea esculenta) is a local food produced by several areas in Indonesia. Few studies have reported its health benefits for glucose-insulin maintenance in diabetes mellitus (DM) but little is understood about its mechanism of action. PGC-1α is a transcriptional co-activator for genes that involved in energy metabolism and increased expression of this gene has previously been associated with improved insulin sensitivity.

Objectives: The objective of this study was to investigate the effect of Lesser yam starch with or without butyrogenic bacteria Eubacterium rectal on PGC-1α expression in skeletal muscle of diabetic rats.

Materials and Methods: Three-month old male diabetic Wistar rats (n_total = 38) were divided into 3 groups based on dietary supplement: lesser yam-starch only (DM Ls); Eubacterium rectal only (DM Bt) and lesser yam-starch with Eubacterium rectal (DM Ls+Bt). Diabetic controls (DM Con) and non-diabetic controls (Non DM Con) groups were used in this study. After 4 weeks of treatment, skeletal muscle tissues were collected from musculus vastus lateralis. Fasting plasma glucose (FPG) was measured before and after treatment. PGC-1α expression was measured with immunohistochemistry and quantified by dividing PGC-1α producing cells with total cells.

Results: After treatment, FPG levels decreased in DM Ls, DM Bt, DM Ls+Bt groups (all p < 0.001) compared to baseline. Additionally, protein expression of PGC-1α in DM Bt and DM Ls+Bt was significantly higher compared to DM Con (p = 0.001 and p = 0.03, respectively).
Conclusion: This study showed that supplementation of lesser yam-starch with butyrogenic bacteria *Eubacterium rectal* was able to improve glucose control in diabetic rats and this effect may be due to PGC-1α activation. Further study is needed to investigate the effect of this treatment in diabetic patients.

P017

Myricitrin Attenuated Insulin Resistance by Regulating Glucose-Activated Transcription Factor Involved in the Development of Metabolic Syndrome in Type 2 Diabetic db/ db Mice

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Objectives: Myricitrin is a rhamnose glycoside of myricetin contained in various plants. Although it is reported to possess antioxidant, anxiolytic and antinociceptive effects, little is known about the effectiveness of myricitrin on glucose metabolism. The purpose of this study was to investigate the effect of myricitrin on glucose metabolism in genetically diabetic db/db mice.

Methods: Twenty male four-weeks-old C57BL/KsJ-db/db mice were randomly divided into two groups and fed an AIN-76 semisynthetic diet (DB group) or AIN-76 semisynthetic diet plus 0.02% myricitrin (MYR group) for 5 weeks.

Results: Final body weight was significantly lower in the MYR group than in the DB group. Although blood glucose concentration was not significantly different between DB and MYR group, level of blood HbA1c, a marker of long-term glycemic control, was significantly lower in the MYR group than in the DB group. Plasma insulin and homeostasis model assessment of insulin resistance levels were also significantly lower in the MYR group than in the DB group. Activities of hepatic gluconeogenic enzymes, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, were significantly decreased in the MYR group compared to the DB group. Moreover, mRNA expression of carbohydrate response element-binding protein, a transcription factor that has been shown to regulate carbohydrate metabolism in the liver in response to elevated glucose concentrations, was decreased in the liver of MYR group compared to DB group.

Conclusions: These results indicate that myricitrin improves glucose metabolism and insulin sensitivity by suppressing hepatic gluconeogenic gene expression and enzyme activities. Thus, myricitrin may have potential as source of anti-diabetic agents to improve insulin resistance.

P018

Modulation of the Association Between PEPD Variant and Risk of Type 2 Diabetes by n-3 Fatty Acids in Chinese Hans

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Objectives: This study sought to determine whether the associations of GWAS-identified genetic variants with risk of type 2 diabetes (T2D) were modulated by n-3 fatty acids in Chinese Hans.

Methods: Six hundred and twenty-two T2D patients and 293 healthy controls were recruited. Erythrocyte phospholipid fatty acids were measured by standard method. Nine GWAS-identified T2D-related single-nucleotide polymorphisms (SNP) were genotyped (rs1436953 at C2CD4A, rs11257655 at CDC123, rs4712524 at CDKAL1, rs2383208 at CDKN2B, rs16955379 at CMIP, rs3786897 at PEPD, rs831571 at PSMD6, rs13266634 at SLC30A8, rs1359790 at SPRY2). These SNPs were all identified in GWAS of Asian populations with high minor allele frequency (>0.2).

Results: Among the nine SNPs, only SNP rs3786897 at PEPD gene showed significant interaction with erythrocyte n-3 fatty acids (P-value after Bonferroni correction = 0.027). No significant association between rs3786897 risk allele (A allele) and higher risk of T2D was observed (GA+AA vs GG: OR = 1.21, 95%CI: 0.84–1.75). The rs3786897-A allele was associated with higher risk of type 2 diabetes (GA+AA vs GG: OR = 2.16, 95%CI: 1.32–3.55) when erythrocyte total n-3 fatty acids were lower than the population median, however, no significant association (GA+AA vs GG: OR = 0.63, 95%CI: 0.35–1.12) was observed when erythrocyte total n-3 fatty acids was higher than the population median.

Conclusions: The association between PEPD genetic variant and risk of type 2 diabetes was modulated by n-3 fatty acids. Higher n-3 fatty acids may abolish adverse effect of risk allele at PEPD for type 2 diabetes.

P019

Diet and Telomere Length in Patients with Cardiovascular Risk: A Systematic Review of the Literature

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Background: Cardiovascular diseases (CVD) remain a leading cause of death worldwide. Leukocyte telomere length (LTL) is a novel indicator for chronic vascular diseases with strong evidence associating shorter telomere length and lower telomerase levels with increase risk.

Objective: To evaluate the evidence for healthy diet and telomere length in subjects with risk of CVD.

Hypothesis: Higher adherence to a healthy diet is related to longer LTL or/and highest telomerase levels.
Methods: A systematic literature search was conducted through Cochrane Library, Scopus, MEDLINE, PubMed and ISI Web of Science databases using MESH terms ‘diet’, ‘food’, ‘nutrients’, ‘telomere length’, ‘cardiovascular disease’ and ‘atherosclerosis’ with no date limitations.

Results: The search strategy resulted in 211 citations, with only 12 meeting the inclusion criteria. Studies associated high intakes of meat, especially processed meat (p = 0.030), and high fat and oils used for cooking (p = 0.037) with shorter LTL. High fruit and vegetable consumption (p = 0.020) and Chinese tea drinking (p = 0.002) attenuated telomere shortening. High total fat intake (p = 0.002), higher linoleic intake (p = 0.001), and elevated total energy intake (p = 0.002) were inversely linked to LTL, whereas dietary fibre intake (p = 0.004) and elevated baseline blood levels of omega-3 fatty acids (p = 0.001) resulted in lower rate of telomere shortening. According to healthy lifestyle factors, improved diet and other modified CVD risk factors were positively associated with longer LTL (p = 0.050) and increased telomerase activity (p = 0.031). Finally, highest adherence to Mediterranean Diet exhibited longer LTL (p = 0.003) and higher telomerase activity (p = 0.013).

Conclusions: Healthy diet appears to be associated with telomere length modulation and reduce predisposition to CVD. The overall quality of the evidence was low due to the novelty of the field suggesting a need for clinical research assessing the role of diet in telomere shortening in adults with risk of CVD to confirm these associations, while isolating diet from other lifestyles factors.

Abstracts

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P020
Increased Placental Leptin Expression is Associated with Low Birth Weight in Malaria-Infected Pregnancy

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Objectives: Infants born with low birth weight (LBW) have a higher risk for developing metabolic diseases in adulthood as a result of fetal programming which modify expression pattern of metabolic hormones regulating placental function and fetal growth. Malaria infection during pregnancy has been associated with LBW, however the subsequent risks for adult metabolic diseases remain unclear. In this study, we investigate the expression of leptin and its receptor (LEP and LEPR) in placenta of malaria-infected pregnant woman who gave birth to infants with appropriate birth weight (birth weight ≥3000 g) (N = 19), inappropriate birth weight (birth weight 2999–2500 g) (N = 14), and low birth weight (birth weight < 2500 g) (N = 11). We hypothesized that the changes of LEP and LEPR expressions are early indication for fetal programing in malaria-associated pregnancy.

Methods: This study employed 44 archived placental biopsies from Plasmodium falciparum-infected pregnant woman in Timika, Papua, Indonesia collected in 2005–2008. Total RNA were extracted from placenta and subjected to reverse transcription PCR for cDNA synthesis. Real time PCR was performed to determine the expression of LEP and LEPR genes relative to GAPDH as a housekeeping gene using TaqMan gene expression assay.

Results: We observed a significantly higher LEP expression in low birth weight group as compared to appropriate and inappropriate birth weight groups (p = 0.0004). However, LEPR expression in all groups showed no significant difference (p = 0.176). We found a significant inverse correlation between placental LEP expression and birth weight (R² = 0.071, p = 0.044). The similar trend was also observed in LEPR expression, although it was not statistically significant (R² = 0.041, p = 0.098).

Conclusion: Changes of LEP and LEPR expression indicated fetal programing in malaria-infected pregnancy.

P021
The Effect of Lesser Yam (Dioscorea esculenta) and Short Chain Fatty Acid-Producing Bacteria on Insulin Receptor Substrate 1 Expression in Skeletal Muscle of Diabetic Rats in Skeletal Muscles of Type 2 Diabetic Rats

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Background: Decreased in insulin sensitivity in skeletal muscle is a major pathogenesis of type 2 diabetes mellitus (T2DM). Short chain fatty acid (SCFA) has been found to have an advantageous effect on insulin sensitivity in T2DM.

Objective: The objective of this study was to examine the beneficial effect of lesser yam (Dioscorea esculenta) and Eubacterium rectale (SCFA-producing bacteria) on insulin receptors (IRS)-1 expression in the skeletal muscles in T2DM rats.

Method: Type 2 diabetes mellitus was induced in adult male Wistar rats by intraperitoneal injection of streptozotocin (STZ) and nicotinamide (NA). 28 rats with diabetes and 7 rats without diabetes were divided into 5 groups. Group 1 was the normal group and Group 2 was the control DM group. Group 3, 4, and 5 were the diabetic rats that received: the lesser yam starch 0.35 g/kg body weight only; 1% Eubacterium rectale only; and yam and Eubacterium rectale, respectively for 4 weeks. IRS-1 expression was measured with immunohistochemistry and quantified by dividing IRS-1 producing cells with total cells.

Result: Administration of yam and Eubacterium rectale reduced blood glucose levels and increased IRS-1 expression significantly in T2DM rats. Blood glucose levels reduced by 22.29%, 43.69%, and 47.00% in Group 3 (203.86 vs. 158.41 mg/dL), Group 4 (212.77 vs. 119.81 mg/dL) and Group 5 (199.12 vs. 105.52 mg/dL), respectively following the 4-week treatments. Furthermore, Group 2 (7.45%) had
lower Irs-1 expression in the skeletal muscles compared to Group 3 (8.77%), Group 4 (19.89%), and Group 5 (16.17%); p < 0.05 for all.

**Conclusion:** These results suggest that there is a synergistic effect of local lesser yam with SCFA-producing bacteria (*Eubacterium rectale*) in improving insulin sensitivity by increasing skeletal Irs-1 expression in T2DM rats.

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**P022**

**Fish Oil Supplementation Attenuates Obesity and Insulin Resistance with No Change in the Inflammatory State Induced by a High Fat Diet in Mice**

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**Introduction:** Fish oil (FO), rich in n-3 polyunsaturated fatty acids, has been shown to regulate body-weight homeostasis but the mechanisms involved are still not elucidated.

**Methods:** C57Bl/6J male mice received a balanced diet (BD) (76% carbohydrate, 9% fat, 15% protein) for four weeks and afterwards a high fat diet (HFD) (26% carbohydrate, 59% fat, 15% protein) for eight weeks. Another group was maintained in a balanced diet for twelve weeks. Fish oil was given (High-EPA Fish Oil, Naturalis, BR) by gavage at 2 g per Kg/body weight, three times per week, during 12 weeks. The following parameters were measured: body weight gain, glycemia, insulinemia, HOMA-IR index, mRNA expression (by RT-PCR) of F4/80, IL-1β, IL-6, TNF-α, TLR-4, TLR-2, adiponectin and leptin in the liver and epididymal fat tissue.

**Results:** HFD increased: body weight gain (3.6-fold), mesenteric (2.6-fold), epididymal (2.7-fold) and retroperitoneal (3.9-fold) fat tissue weights, plasma total cholesterol (1.3-fold) and LDL-cholesterol (1.3-fold) levels, glycemia (1.2-fold), insulinemia (1.6-fold), HOMA-IR index (2.6-fold), percentage of fat in the liver (2.5-fold), mRNA expression of F4/80 (3.5-fold), IL-1β (2.2-fold), TNF-α (3-fold) and leptin (2.7-fold) in epididymal fat tissue as compared to BD. HFD did not change mRNA expression of inflammatory proteins in the liver. FO supplementation for twelve weeks decreased: body weight gain (23%), mesenteric (41%) and epididymal (29%) fat tissue weights, plasma total cholesterol (30%) and LDL-cholesterol (36%) levels, HOMA-IR index (24%) and the percentage of fat in the liver (21%) in the HFD mice. FO supplementation did not change mRNA expression of inflammatory proteins in the liver and epididymal fat tissue of the HFD group.

**Conclusion:** HFD caused obesity, insulin resistance and adipose tissue inflammation. FO supplementation attenuated obesity and insulin resistance but did not change the inflammation state of the epididymal fat tissue.

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**P023**

**Fish Oil Supplementation Modulates Expression of Genes Associated with Insulin Signaling, Glucose and Lipid Metabolism, Mitochondrial Function and Redox State in Skeletal Muscle from Mice Fed a High-Fat Diet**

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Obesity and insulin resistance induced by high-fat diet (H) have been associated to alterations in expression of genes involved in glucose and lipid metabolism and, lately, also to reduced mitochondrial mass and activity. Omega-3 polyunsaturated fatty acids (n-3 PUFA) have been shown to improve insulin sensitivity and glucose homeostasis in rodent models of insulin resistance, however the mechanisms involved remain unknown. In a previous study, we showed that supplementation with fish oil (FO), a source of n-3 PUFA, reduces oxidative stress and increases basal O2 consumption and the content of tricarboxylic acid cycle intermediates (citrate, α-ketogluarate, malate, oxaloacetate) in skeletal muscle. The effects of FO on expression of genes associated with insulin signaling and energy metabolism were investigated in insulin-resistant mice. C57Bl/6 male mice (8 wks-old) were divided into three groups: control diet (C), high-fat diet (H) and H + FO (HFO). FO was given by oral gavage (2 g/Kg b.w.), three times per week, during 12 weeks. Total RNA was extracted from soleus muscles and gene expression evaluated by Real-Time PCR. High-fat diet decreased expression of IRS1, PEPCK, PGC1α, PPARα, UCP3, and antioxidant enzymes SOD2 and GPX1. FO supplementation upregulated GPAT1 and PEPCK and mitochondrial proteins expression (CPT1, PGC1α, PPARα and UCP3) in mice fed H diet. However, FO had no effect on expression of IRS1, SOD2 and GPX1. Expressions of GLUT4, DGAT1, PLIN5, antioxidant enzymes and mitochondrial proteins involved in oxidative phosphorylation (NDUFB3, NDUFB5, SDHB, SLC25A12, CYC1 and SURF1) were not changed by H diet or FO. These results suggest that n-3 PUFA abolishes insulin resistance in skeletal muscle by modulating expression of genes associated to energy metabolism.
P024

ω-3 Polyunsaturated Fatty Acid Docosahexaenoic Acid (DHA) Reduces the Inflammatory-Mediated Up-Regulation of Phosphodiesterases (PDE)5A in Human Vascular Endothelium

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Objectives: PDE5A is a phosphodiesterase that catalyses the hydrolysis of cGMP into GMP, thus curtailing NO signalling and favouring pro-angiogenic effects. The ω-3 fatty acids DHA and eicosapentaenoic acid (EPA) are considered health-promoting nutrients. However, molecular mechanisms underlying their effects remain incompletely understood. We therefore decided to investigate whether inflammatory stimuli known to be involved in the inflammatory-mediated endothelial dysfunction and angiogenesis affect endothelial PDE5A expression, and whether and how cell exposure to DHA modifies such expression.

Methods: Human umbilical vein endothelial cells (HUVEC) were treated with increasing concentrations of inflammatory and pro-angiogenic stimuli (interleukin (IL)-1, tumour necrosis factor (TNF) α, IL-6, and vascular endothelial growth factor (VEGF)) for 0–24 hours. After this time, PDE5A protein and mRNA expression were assessed by Western and qPCR, while the activation of Nuclear factor (NF)-κB and Activator Protein (AP)-1, in terms of RelA-, c-Fos-, pphospho-c-Jun-, Fos-B-, and Fra-1-DNA-binding, were assessed by trans-activation assays. To evaluate DHA effect on PDE5A expression, HUVEC were treated with 0–50 μmol/L DHA for 48 hours before stimulation.

Results: PDE5A protein and mRNA expression increased significantly only after stimulation with 10 ng/mL IL-1β and TNFα (P < 0.01 vs control). DHA treatment of HUVEC for 48 hours before stimulation reduced PDE5A induction at protein and mRNA level (P < 0.01 vs control). DHA reduced PDE5A induction negatively interfering with both NF-κB and AP-1 activation. Since PDE5A inhibitors are now approved for use in erectile dysfunction and pulmonary hypertension and have a potential in treating other disease states featuring endothelial dysfunction, downregulating the inflammation-mediated expression of PDE5A DHA may positively impact the NO/sGC/cGMP axis and reproduce the therapeutic potential of PDE5 inhibitors.

P025

Gene Network and Canonical Pathway Analysis of Epididymal Adipose Tissue in High-Fat Diet Induced Obese Mice

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Objectives: Obesity is characterized by increased adipose tissue mass that results from both hyperplasia (increased fat-cell number) and hypertrophy (increased fat-cell size). It alters adipose tissue metabolic and endocrine function and leads to an increased release of fatty acids, hormones, and pro-inflammatory molecules that contribute to obesity-associated complications. Since obesity occurs as a result of an interaction between a number of genes and environmental factors such as diet, aim of the present study was designed to identify hub genes and core molecular networks of epididymal adipose tissue in response to high fat diet (HFD)-induced obesity.

Methods: Male C57BL/6J mice (4-week-old) were fed a normal diet (ND) and HFD (60% kcal from fat) for 12 weeks. Global gene expression profiles of epididymal adipose tissue were analyzed using mRNA sequencing, and differentially expressed genes (DEG) were filtered based on a selection criterion (fold-change ≥ 2, p-value < 0.05). Top networks and canonical pathways associated with sets of specific genes of interest were constructed using bioinformatics tools, Ingenuity Pathways Analysis (IPA).

Results: The mRNA sequencing analysis identified 1119 genes (1067 up-regulated genes and 52 down-regulated genes). Analysis using IPA software revealed three top-scoring networks in epididymal adipose tissue significantly modified by HFD (417 molecules were involved in these networks): nutritional disease (Network 1), metabolic disease (Network 2), and lipid metabolism (Network 3). When we merged these top-scoring networks, ADAM8, CFD, MMP12, ATF3, and AGT appear to be hub genes integrating molecular interactions associated with obesity. The top canonical pathways included Fcg receptor-mediated phagocytosis in macrophages and monocytes, CD28 signaling in T helper cells, leukocyte extravasation signaling, agranulocyte adhesion and diapedesis, and granulocyte adhesion and diapedesis.

Conclusions: Our data provides candidate genes and associated molecular networks of epididymal adipose tissue involved in the development of diet-induced obesity, which may be helpful in better understanding of obesity as well as identifying new therapeutic targets and diagnostic biomarkers of obesity.
Global Changes in Hepatic Transcriptional Profiles in Response to Green Tea in Diet-Induced Obese Mice

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Objectives: Green tea is well known to have beneficial effects on obesity and hepatic steatosis. However, changes in transcriptional profiles in response to green tea are little known and needs to be elucidated. In the present study, we investigated the effect of Green tea on plasma and hepatic lipid profiles and hepatic lipid metabolism-related-gene expression using mRNA sequencing analysis in high-fat diet (HFD) fed mice.

Methods: Twenty male C57BL/6J mice (4-week-old) were randomly divided into 2 groups and fed HFD (60% kcals from fat) or HFD supplemented with 0.25%(w/w) green tea extract (GT) for 12 weeks.

Results: After 12 weeks supplementation of GT, body weight, body weight gain, liver weight and adipose tissue weight were significantly decreased. The supplementation of GT improved not only plasma lipid profile such as total cholesterol and HDL-cholesterol but also remarkably decreased hepatic total cholesterol content. Green tea extract also led to increase leptin and resistin concentrations. mRNA sequencing analysis revealed that GT down-regulated of hepatic expression genes that is involved in lipid metabolism, such as PPAR signalling and fatty acid metabolism.

Conclusions: Our findings suggest that Green tea extract supplementation attenuates hepatic steatosis by decreasing hepatic PPAR signalling and altering hepatic fatty acid metabolism-related genes.

Plasma Micronutrient and Mineral Profile (Plasma Nutriome) of Prostate Cancer Cases Is Altered Relative to Healthy Controls – Results of a Pilot Study

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Objectives: Emerging evidence suggests the possible role of various micronutrients in cancer prevention. The present study was designed to test the hypothesis that the plasma levels of different micronutrients and trace elements profile in prostate cancer patients is different from that of healthy controls.

Methods: Plasma samples from 116 Caucasian men affected with late onset of prostate cancer and 132 matched controls from South Australian population were collected and analysed for their concentration of micronutrients and trace elements.

Results: Plasma concentration of lutein, lycopene and carotenoids (alpha and beta) were found to be significantly lower in prostate cancer patients (p = 0.02, 0.008, 0.002 and 0.002 respectively). Plasma levels of trace elements such as iron, copper, calcium and sulphur were significantly higher (p < 0.0001, < 0.0001, < 0.0001 and p = 0.0003 respectively) while that of selenium was significantly lower (p = 0.02) in prostate cancer patients. Prostate cancer risk was significantly associated with low plasma levels of lycopene (OR: 2.0; 95% CI: 1.15–3.47) and β-carotenoid (OR: 1.89; 95% CI: 1.09–3.26) and high levels of iron (OR: 1.94; 95% CI: 1.1–3.44), calcium (OR: 3.65; 95% CI: 1.9–7.02) and sulphur (OR: 1.96; 95% CI: 1.11–3.46).

Conclusions: Our findings that the micronutrient and mineral profile in plasma (i.e. plasma nutriome) could be a useful diagnostic of prostate cancer risk.

Folate Transport and Metabolism Gene Polymorphism and Prostate Cancer Risk in South Australia

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Objectives: Single nucleotide polymorphisms (SNPs) in folate transportation and metabolism pathway genes such as 5’, 10’-methylene-tetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR), glutamate carboxypeptidase II (GCPII), reduced folate carrier (RFC), thymidylate synthase (TS), transcobalamin II (TCII), cystathionine β-synthase (CBS) can lead to alterations in the metabolism and transportation of folates and in the synthesis of S-adenosyl-methionine (SAM), the most active methyl donor in the body. This could play an important role in carcinogenesis through altered DNA methylation and nucleotide synthesis.

Methods: To elucidate the role of these SNPs in these genes, we conducted a case-control study comprising of 116 Caucasian men affected with the late onset of prostate cancer and 132 matched controls from South Australia.

Results: Prostate cancer risk was significantly increased for MTHFR 677CT/TT (OR: 2.19; 95% CI: 1.29–3.72) but significantly reduced for MTHFR 1793AG/GG (OR: 0.42; 95% CI: 0.25–0.71) and for GCPII 1561CT/TT (OR: 0.25–0.71) and and for GCPII 1561CT/TT (OR: 0.12; 95% CI: 0.05–0.28) and for GCPII 1561CT/TT (OR: 0.12; 95% CI: 0.05–0.28).

Conclusions: Our results suggest that common SNPs in genes such as MTHFR (C677T and A1793G) and GCPII (C1561T) involved in the folate metabolism pathway contribute to prostate cancer susceptibility.
Geraniin Selectively Induces High Rates of Chromosomal Instability and Apoptosis in Human Colorectal Adenocarcinoma Cell Lines


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Objective: Geraniin is a main polyphenolic component of several genuses such as Geranium and Phyllanthus. It was reported that geraniin induces chromosomal instability (CIN) in human cancer and noncancer cells.

Methods: The cytokinesis-block micronucleus cytome assay was employed to evaluate the CIN and cell death induced by geraniin in human colorectal adenocarcinoma cell lines, Colo205, Colo320 and normal colon mucosal epithelial cell line NCM460. Cell were exposed to geraniin (25–100 μg/ml) dissolved in RPMI-1640 for 24, 48, 72, or 96 hours. Cells were harvested and scored to determine the frequencies of the nuclear division index (NDI), apoptosis, necrosis, binucleated cells with micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBuds).

Results: The results showed that geraniin significantly reduced the NDI and necrosis, increased CIN (MN, NPB, NBuds) and apoptosis both in Colo205 and Colo320 with a dose-dependent manner (p < 0.001). However, the chemical significantly increased the NDI, decreased the CIN, apoptosis and necrosis in NCM460 cells with a dose effects (p < 0.001). These findings indicated that geraniin induces CIN and apoptosis in colorectal adenocarcinoma cells, whereas protects against CIN and apoptosis in noncancer cells at the tested doses. Considering the cancer cell lines, Colo320 possesses higher genetic damage background and lower apoptosis rate compared to Colo205 (p < 0.001). However, Colo320 showed less significant increase in CIN but more significant increase in apoptosis compared to Colo205 after geraniin treatment (p < 0.001).

Conclusions: The research indicated that geraniin has some anti-cancer potential by eliciting apoptosis-inducing effect in cancer cells with elevated basal level of CIN. Geraniin didn’t show adversely effects on non-cancer cells and should be useful in cancer treatment.

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Feijoa Inhibits Toll-Like Receptor Signalling and Activates Autophagy Implicating a Role in Dietary Control of IBD

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Background: Dietary intervention has shown promise in the better management of Inflammatory Bowel Disease (IBD). In this study we took a pathway specific approach to understand how fruits and in particular feijoa, interact with a relevant pathway associated with IBD risk. Here we investigated components of the bacterial sensing pathway including pattern recognition receptors (PRR) and autophagy.

Methods: fractionated fruits were screened for their anti-inflammatory properties. A feijoa fraction was selected to study further based on promising screening results. Finally, the PRR-activated inflammatory and autophagic response was measured in intestinal epithelial cell lines (IECs).

Results: Findings suggest that the feijoa fraction interacts with the bacterial sensing system in IECs. The feijoa fraction appears to work with the cell’s response system towards bacterial ligands. This fraction mainly works through a TLR2/1 specific mechanism and may be linked to autophagy.

Conclusion: These findings are promising and suggest a dynamic role of dietary extracts when it comes to interacting with the cell’s response to the external environment. Further understanding of these interactions has the potential to help in the development of personalized nutrition and better management of IBD.

Influence of Ximenynic Acid on Cell Cycle Arrest and Apoptosis of HepG2 Cells

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Objectives: To investigate whether ximenynic acid can induce apoptosis and cell cycle changes of human liver cancer cells (HepG2). Further, to disclose the underlying mechanisms of the anticancer effect of ximenynic acid with several molecular biological methods.

Methods: Oil Red O, MTT assay, FCM (flow cytometry), RT-PCR, and western blotting analyses were applied to investigate the effects of ximenynic acid on HepG2 cells.

Results: Increased lipid accumulation appeared in HepG2 cells treated with ximenynic acid and oleic acid. MTT assay and FCM analysis prove that ximenynic acid can inhibit proliferation and
induce apoptosis of HepG2 cells in a dose dependent manner. After treating HepG2 cells with ximenynic acid, we found that the proportion of cells in G0/G1 phase increased significantly (P < 0.01). Accordingly, the percentage of cells in G2/M and S phases decreased. RT-qPCR results indicated that the expression of COX-1 was obviously inhibited by ximenynic acid along with the decrease of cell cycle mRNAs CCND3 and CCNE1 expression. Western blotting analyses showed that the expressions of proteins involved in apoptosis regulation including NF-xB, PARP, Caspase 3 were down-regulated in the ximenynic acid-treated groups.

Conclusions: Ximenynic acid can be absorbed by HepG2 cell as oleic acid. It can prevent cells to enter G2/M and S phases from G1 phase, which is consistent with the decrease of CCND3 and CCNE1 expression. Furthermore, it inhibits proliferate and induces apoptosis of HepG2 cells, which may due to the decreased expression of COX-1 mRNA and down-regulation of NF-xB, PARP, Caspase 3 proteins.

P032
Effects of Dietary Fiber on Inflammation-induced Colorectal Carcinogenesis in Mice

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Objectives: The aim of present study was to investigate the effects of konjac glucomannan (KGM), inulin and its combination (K+ I) on the T lymphocyte profile and cytokines level in the Peyer’s patch, intestinal intra-epithelium and lamina propria lymphocytes isolated from, gut associated lymphoid tissues (GALT), in colonic tumorigenesis mouse model induced with azoxymethane (AOM) and dextran sulfate sodium (DSS).

Methods: Male C57BL/6J (n = 40; 6 weeks old) mouse were given an intraperitoneal injection of AOM (10 mg/kg BW) followed by three cycle of 1–1.5% DSS in drinking water to induce colonic tumorigenesis. After the 9-week dietary intervention, the T lymphocyte profile and Thl/2 cytokines in the GALT were analysed with flow cytometry and commercial ELISA kit, respectively.

Results: Dietary supplementation with all soluble fibers enhanced the cytotoxic T (CD3+CD8+) cells (% total GALT cells), and K+ I increased the helper T (CD3+CD4+) cells (% total GALT cells), as compared to that of control counterpart, respectively. However, dietary fiber supplementation did not change total T cells (% total GALT cells). The production of pro-inflammatory cytokine (Th1 type) tumor necrosis factor alpha and interlukin (IL)-6 were significantly lower in all fiber-supplemented groups than that in the control group. The RT-qPCR results indicated that the expression of COX-1 was obviously inhibited by ximenynic acid along with the decrease of cell cycle mRNAs CCND3 and CCNE1 expression. Western blotting analyses showed that the expressions of proteins involved in apoptosis regulation including NF-xB, PARP, Caspase 3 were down-regulated in the ximenynic acid-treated groups.

Conclusions: The present study indicated that soluble fibers, KGM and inulin could regulate the inflammatory responses in colorectal tumorigenesis induced by AOM/DSS by enhancing sub-population of cytotoxic and helper T lymphocytes and reduced the Th1 type pro-inflammatory cytokine levels in the GALT.

P033
Vegetable Intolerances: The rs12212067 FOXO3-Diet Interaction in CD Patients

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Background: Diet is known to play a major role in Crohn’s disease (CD), it has also been reported that the minor G allele from rs12212067 polymorphism (T>G) in FOXO3 is associated with milder CD. We investigated the association between the rs12212067 polymorphism and vegetable consumption.

Methods: A total of 44 vegetable intolerances were recorded on a self-reported dietary questionnaire. Each vegetable was scored on a 5-point ordinal scale: responses for ‘make no difference’ or ‘don’t know’ were not utilised in the analysis. Beneficial effects were responded as ‘++’ or ‘+’ and adverse effects were responded ‘–’ or ‘−’.

Results: Our results showed that the minor G allele significantly and adversely associated with consuming cooked tomato, raw tomatoes, Chinese greens and baked beans in CD patients.

Conclusions: CD patients carrying the G allele in the rs12212067 FOXO3 SNP have an increased risk of adverse symptoms if they eat cooked tomato, raw tomatoes, Chinese greens or baked beans. These patients should be advised to avoid eating these vegetables.

P034
Variation in the BCMO1 Gene and Circulating Levels of Carotenoids in Different Brazilian Ethnic Groups

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β-carotene, the most abundant provitamin A carotenoid in the diet, is converted to retinol by β-carotene 15,15'-monooxygenase (BCMO1). Because of genetic variability in β-carotene metabolism it absorption and conversion into retinol is extremely variable among individuals. The aim of this study was to investigate the effects of the single nucleotide polymorphisms (SNPs) A379V:rs7501331 and R267S:rs12934922 from the BCMO1 gene on β-carotene conversion efficiency in two Brazilian ethnic groups: 31 African ancestry and 45 European ancestry were analyzed. Genotyping of polymorphic variants was performed by real-time polymerase chain reaction. Analysis of retinol esters and carotenes were performed using a Dionex HPLC
system and the statistical analyses were performed using SPSS 17.0. Allele frequencies for the A and T allele of rs12934922, for African and European ancestry, were 71.0 and 60.0% and 29.0 and 40.0%, respectively. Likewise the allele frequencies for the C and T allele of rs7501331 were 85.5 and 71.1% and 14.5 and 28.9%, for African and European ancestry, respectively. The current study indicated that β-carotene and retinol concentrations were significantly positively correlated with genotype TT of rs12934922 in African ancestry (r = 1; P < 0.01) and negatively correlated with rare genotype TT of rs7501331 in European ancestry (r = -1.00; P ≤ 0.01). Thus we can hypothesize that subjects with the allele T, specifically the genotype TT, are poor responders to dietary β-carotene. For African ancestry we were able to found a decrease tendency for β-carotene and retinol conversion in genotype rs12934922, whereas this is more pronounced for European ancestry retinol levels in genotype rs7501331. We showed that two common non synonymous SNPs are present in the VDR-Tru91 wildtype (UU) genotype. As expected, a positive association between dietary intake of vitamin D and calcium with respect to osteoporosis was observed, a likely result of neutraceutical intervention. Further studies should help elucidate VDR gene variants as potential markers of osteoporosis risk, and any contributing effect of dietary intake.

**P035**

**Vitamin D Receptor Genetics and Calcium Intake in an Elderly Australian Cohort with Osteoporosis**

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**Objectives:** Osteoporosis is a degenerative condition with a gradual but progressive decline in bone density, resulting in rarefied bone tissue. Genetic polymorphisms in the vitamin D receptor (VDR) appear to influence vulnerability to bone fragility, disability and deformity, revealing a growing importance for the VDR in bone disorders. We investigated the association between dietary vitamin D and calcium intake, and incidence of osteoporosis, with respect to eight VDR gene variants in an elderly Australian cohort.

**Methods:** 608 subjects (287 males; 363 females; mean age 78 years) were recruited from the Central Coast, NSW. Subjects provided fasting blood samples and completed a self-administered FFQ and medical history. Genotype analysis was performed using PCR-RFLP. Diet intake was analysed using FoodWorks™ database (Version 9). Stepwise and nominal logistic regression analyses were carried out.

**Results:** VDR-Tru91 was significantly associated with osteoporosis in females (p = 0.0046; r² = 0.0864). A significant association between osteoporosis and total dietary intake of vitamin D (p = 0.0009; r² = 0.0708; slope = -0.0007) and calcium (p = 0.0003; r² = 0.0708; slope = -0.0341) was found for VDR-Tru91 (wildtype genotype UU). Independent of VDR genotype, both dietary intake of vitamin D and calcium were associated with osteoporosis (p ≤ 0.0001), with distribution of intake showing higher mean values for vitamin D and calcium in subjects with osteoporosis (11.72 µg/d; 1307.86 mg/d) compared with subjects without (6.15 µg/d; 1017.00 mg/d).

**Conclusions:** VDR-Tru91 was associated with risk for osteoporosis in females, and dietary vitamin D and calcium intake and the incidence of osteoporosis was strongly associated with the VDR-Tru91 wildtype (UU) genotype. As expected, a positive association between dietary intake of vitamin D and calcium with respect to osteoporosis was observed, a likely result of neutraceutical intervention. Further studies should help elucidate VDR gene variants as potential markers of osteoporosis risk, and any contributing effect of dietary intake.

**P036**

**The Effect of Lesser Yam (Dioscorea esculenta) Flour on Adenosine Monophosphate-Activated Protein Kinase-α2 (AMPK-α2) Expression in Liver and Skeletal Muscle of Streptozotocin (STZ) and Nicotinamide (NA)-Induced Diabetic Rats**

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**Background:** Diabetes mellitus is an endocrine disease characterized by hyperglycemia. Lesser yam (Dioscorea esculenta) activates AMPK-α2 through butyrate as a secondary metabolite of inulin and resistant starch fermentation in digestive tract. Adenosine monophosphate-activated protein kinase-α2 (AMPK-α2) is considered a master switch that regulates glucose and lipid metabolism. Reduced activity of AMPK-α2 is associated with insulin resistance resulting in hyperglycemia.

**Objective:** To investigate the effect of lesser yam flour on AMPK-α2 expression in liver and skeletal muscle of streptozotocin (STZ) and nicotinamide (NA)-induced diabetic rats.

**Methods:** Twenty five male Wistar rats were divided into 5 groups equally: normal control (NC); diabetic control (DC); and 3 diabetic groups each treated with 1.25 grams (DLy-1.25), 2.5 grams (DLy-2.5) and 5.0 grams (DLy-5.0) of lesser yam flour. The lesser yam flour was incorporated into the rats’ diet. Fasting plasma glucose (FPG) levels were measured after 5 days of induction and after 4 weeks of intervention. Liver and skeletal muscle tissues were collected and fixed in formalin and embedded in paraffin. Expression of AMPK-α2 was examined with immunohistochemistry.

**Results:** Administration of lesser yam flour to diabetic rats resulted in a significant decrease in FPG levels compared to DC group. Expression levels of AMPK-α2 in liver and skeletal muscle were higher among the lesser yam groups compared to the DC group. AMPK-α2 was highly expressed in the skeletal muscle compared to liver although statistically insignificant. There was no correlation between dosages of lesser yam flour and AMPK-α2 expression lev-
els. Among the lesser yam flour groups, the highest AMPK-α2 expression level was found in the DLy-1.25 group in both liver and skeletal muscle (24.42% and 14.40%, respectively).

**Conclusion:** These results suggest that lesser yam flour has the potential to increase AMPK-α2 expression in liver and skeletal muscle, and thus helps maintain glucose-insulin homeostasis of diabetic rats.

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**P037**

**Epigenetic Regulation of IL6 (In Breast Cancer Cell Lines)**

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**Objectives:** IL6 is a pluri-potent cytokine with metastatic potential in various cancer cell lines; it is highly expressed in the aggressive, metastatic breast cancer cell line MDA-MB231, as compared to the non-aggressive breast cancer cell line MCF-7.

**Methods and Results:** we analysed the transcription factor profile by EMSA and Western analysis, and found that the major difference between the 2 cell lines is due to the different chromatin environment in the proximal IL6 promoter area, which is covered by nucleosomes in MCF-7, and shows an open configuration in the metastatic cell line(s) allowing abundant IL6 expression. The chromatin configuration was analysed by the 'indirect labeling method'. The transition from one configuration to the other can be influenced by natural products.

**Conclusions:** it is possible to control the genomic expression by a healthy diet and life-style.
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