

CARING & SHARING OF DATA IN A COLLABORATIVE PROJECT

Pieter Demmers¹ and Alan McCulloch², Crop & Food Research¹ Invermay, Dunedin
Agresearch² Invermay, Dunedin.

The Nutrigenomics New Zealand (NuNZ) Crohn's Study is a collaborative project between four organisations with multiple locations within New Zealand. This study produces data from several disciplines including food component, genotype, physiological and clinical, and gene and protein expression data and therefore poses challenges of data storage, analysis and information sharing.

The Nutrigenomics Database is being developed using open-source platforms and techniques to provide central, secure storage of the data accessible via the web by all of the participating scientists enabling different views of data and as a tool for cross experimental and disciplinary analysis.

A Wiki ("NutriWiki") has been implemented to enable a central point for sharing information (such as experiment protocols, data formats, project plans and meeting minutes) and as a forum for ideas and discussion.

The current status of the NuNZ database and NutriWiki is presented and future plans for integration with other databases and development of analysis tools are outlined.

All of the studies described were approved by the University of Auckland Animal Ethics Committee. Supported by the HRC, the Marsden Fund and the University of Auckland Research Committee

Applying statistics to high dimensional systems

Page GP¹, Zakharkin SO¹, Kim K¹, Wang J¹, Loraine A¹, Cui XQ¹, Mehta T¹, Wei H¹,
Allison DB², Barnes SB¹

Departments of Biostatistics¹, Pharmacology and Toxicology² University of Alabama at
Birmingham

In just a few years, microarrays have gone from obscurity to being almost ubiquitous in biological research. At the same time, the statistical methodology for microarray analysis has progressed from simple visual assessments of results to a weekly deluge of papers that describe purportedly novel algorithms for analyzing changes in gene expression. Although the many procedures that are available might be bewildering to biologists who wish to apply them, statistical geneticists are recognizing commonalities among the different methods. Many are special cases of more general models, and points of consensus are emerging about the general approaches that warrant use and elaboration. These points will be highlighted by our work in the area of power, quality control, inference and data mining of microarray expression data.

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NUTRIGENOMICS AND CARDIOVASCULAR DISEASES

J.M. Ordovas. USDA-HNRCA at Tufts Univ. Boston, MA. USA.

Objective: Changes in diet are likely to reduce cardiovascular disease (CVD), but after decades of active research and heated discussion the question still remains: what is the optimal diet to achieve this elusive goal? Is a low fat, as traditionally recommended by multiple medical societies? Or a high monounsaturated fat as predicated by the Mediterranean diet? Perhaps a high polyunsaturated fat based on the cholesterol lowering effects? The right answer may be all of the above but not for everybody. A well-known phenomenon in nutrition research and practice is the dramatic variability in interindividual response to any type of dietary intervention.

Methods: There are many other factors influencing response, and they include, among many others, age, sex, physical activity, alcohol, and smoking as well as genetic factors that will help to identify vulnerable populations/individuals that will benefit from a variety of more personalized and mechanistic based dietary recommendations. This potential could and needs to be developed within the context of nutritional genomics that in conjunction with systems biology may provide the tools to achieve the holy grail of dietary prevention and therapy of CVD. This approach will break with the traditional public health approach of “one size fits all.”

Results: The current evidence based on a few candidate genes involved in lipid metabolism (i.e., APOA1, APOA5, APOE, LIPC)[1] has begun to identify subgroups of individuals who benefit more from a low fat diet, whereas others appear to benefit more from a high monounsaturated or polyunsaturated fat (PUFA) diets. Of interest is the increasing evidence showing that when it comes to cardiovascular health, n-6 and n-3 families of PUFAs interact very different with genetic variants to modulate cardiovascular risk factors. Thus, while some subgroups of individuals may be at higher risk from high consumption of PUFA n-6, others may benefit from increased consumption of PUFA n-3. Moreover, given the emerging epidemic of obesity, it is important to understand the effect of obesity in triggering the associations between genetic variants and cardiovascular risk factors (i.e., APOE, APOA5) [2] and the role of genetic variants predisposing to obesity and response to dietary treatments (i.e., PLIN).

Conclusions: The continuous progress in Nutrigenomics will allow us in the future to identify those persons for whom diet plays no major role in their risk of CVD as well as those persons who may benefit from specific gene-based dietary advice.

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GENETIC STUDIES OF CROHN'S DISEASE

Ivonne Petermann, Claudia Hübner, Martin Philpott, Brian Browning, Lynn Ferguson and Andrew Shelling,
Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Inflammatory bowel disease (IBD) is a complex disorder characterised by chronic inflammation of the gastrointestinal tract. There are two main clinical subtypes, Crohn's disease and ulcerative colitis. Crohn's disease can affect any part of the intestine, and is associated with discontinuous, transmural lesions of the gut wall. Ulcerative colitis inflammation is confined to the colon and rectum, and lesions are continuous and superficial. Most studies support a polygenic model of inheritance, and it is clear that environmental factors contribute significantly to the development of the disease. The CARD15/NOD2, DLG5, SLC22A4 and SLC22A5 genes have recently been identified as being involved in the development of Crohn's disease. However, other susceptibility genes must exist and be associated with predisposition to Crohn's disease. It is the major aim of Nutrigenomics NZ to determine how foods and food components affect health at the molecular genetic level by using nutritional genomic methods. Identifying SNPs involved in the development of IBD is an important first step in the Nutrigenomics research programme.

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Using cell-based assays to aid in the development of new functional foods: tracking anti-inflammatory activities in fruit extracts

A Adaim, J Zhang, W Smith, R Wibisono, D Lauren, R Stanley and M A Skinner

The Horticulture and Food Research Institute of New Zealand, Auckland, NZ

Phenolic phytochemicals are abundant micronutrients in fruit and vegetables. There is an emerging body of evidence regarding their health benefits, some of which may be associated with their anti-inflammatory properties. Cell-based assays can provide preliminary evidence for the health benefits of phytochemicals and help to determine the active components in fruit and vegetables. The aim of this work is to track the anti-inflammatory activity of processed apple extracts to the signature molecules that are responsible for the activity. The anti-inflammatory activity exhibited by two apple extracts was measured by the inhibition of production of an inflammatory mediator, tumour necrosis factor- α (TNF- α), from a macrophage cell line. Total phenolics were measured by the Folin-Ciocalteu method. The two apple extracts were fractionated into 12 fractions using LH-20 resin, and the anti-inflammatory activity, total phenolics and HPLC profiles were measured in each fraction. Anti-inflammatory activity was detected in a proportion of the fractions and some of these active fractions displayed higher activity than the parent extract. In some cases anti-inflammatory activity was correlated with total phenolic concentration, while in others it was not associated with fractions high in polyphenols. Further characterisation of the compounds in the active fractions will be carried out using LC-mass spectrometry. This type of in-depth component and bioactivity analysis will be useful for tailoring processed fruit extracts for functional foods.

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MAKING NUTRIGENOMICS ACCESSIBLE TO STUDENTS AND TEACHERS: FOOD FOR THOUGHT

Catherine M. Bunting, Barbara E. Ryan and Sara A. Loughnane, Centre for Science and
Technology Education Research, University of Waikato

Abstract

Nutrigenomics has the potential to impact people of all ages, but societal acceptance relies on education and discussion about the risks and advantages.

Although the concept of nutrigenomics can be taught in the technology, science, and biology school curricula, teachers find “biotechnology” a difficult topic area, and many schools simply opt out of teaching it (McGee et al., 2002). In particular, relevant, up-to-date resources are needed to enhance the understanding of both teachers and their students (Jones, 2004). The New Zealand Biotechnology Learning Hub was established to address this need. In particular, an online platform (www.biotechlearn.org.nz) provides multimedia case studies of biotechnology in action, making modern research projects like nutrigenomics more accessible to school students, teachers and the wider community. The Hub has also responded to the call for the integration of expertise of scientists and educators (Davenport, Hardman, & Savage, 1986; France & Bolstad, 2006), and development of the content is the result of collaboration between these groups.

Students bring knowledge of foods, the digestive system, health and genetics to the classroom, and are keen to learn more. They are excited to discover through the Hub that there is potential for creating a diet especially designed to optimise their individual health and performance needs. The Hub thus provides an excellent learning tool which has the potential to make nutrigenomics better understood by a more general audience.

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DO FLAVONOIDS PROTECT AGAINST ULCERATIVE COLITIS?

Yvonne Dommels, Christine Butts, Ishwani Singh, Sheridan Martell and Julian Heyes,
Crop & Food Research, Palmerston North, New Zealand

Ulcerative colitis is a disease that causes inflammation in the intestine, particularly in the colon. Food components may prevent the occurrence and the extent of this inflammatory response in the intestine. The aim of our study is to test the anti-inflammatory response of some food flavonoids that have been reported to reduce intestinal inflammation. Multiple drug resistance *mdr1a*^{-/-} knockout mice, that spontaneously develop ulcerative colitis because of a functional defect in an ATP dependent transporter, are used as an *in vivo* model of inflammatory bowel disease. Forty-eight 6.5 weeks old *mdr1a*^{-/-} and forty-eight age-matched FVB control mice were obtained from Taconic (USA). All mice were housed individually under conventional housing conditions. Twelve *mdr1a*^{-/-} and twelve FVB mice were either fed an AIN-76 control diet or the AIN-76A diet supplemented with either 0.2% curcumin, 0.1% rutin or 0.6% green tea polyphenol extract for 12 weeks. Fresh diets were fed ad libitum and food consumption was measured. All mice were weighed three times a week and weight loss, stool consistency, rectal bleeding and mobility was monitored and scored using a disease activity index. At 18.5 weeks of age, intestinal samples were collected for histology (inflammatory cell infiltrate, tissue destruction, tissue repair), microarray analysis of gene expression changes, proteomics and enzyme activity of myeloperoxidase, a marker of inflammation. Plasma samples were collected for cytokine analysis. Curcumin, rutin and green tea polyphenol and metabolite levels will be measured in urine, faeces and plasma. Urine samples were collected for metabolomics studies. Thymus, liver, spleen, kidneys and skeletal muscle were collected and stored for subsequent analysis. Overall, using nutritional genomics methods, our study will generate insight into the potential of flavonoids to beneficially affect gut health.

Nutrigenomics New Zealand is a collaboration between AgResearch Limited, Crop & Food Research, HortResearch and The University of Auckland and is largely funded by the Foundation of Research, Science and Technology (FRST).

Using Toll-like receptor ligands to target foods to inflammatory pathways dysregulated in Crohn's disease

Davanea Forbes^{1,4}, Denis Lauren^{3,4}, Kevin Sutton^{2,4}, Carol Wu^{3,4}, Jessie Li^{2,4}, and Margot Skinner^{1,4},

¹*HortResearch, Private Bag 92169, Auckland,* ²*New Zealand Institute for Crop & Food Research Ltd, Private Bag 4704, Christchurch,* ³*HortResearch, Private Bag 3123, Hamilton,* ⁴*Nutrigenomics New Zealand, www.nutrigenomics.org.nz. email: dforbes@hortresearch.co.nz*

Crohn's disease, a chronic inflammatory disease of the gastrointestinal tract, is the initial target of the Nutrigenomics New Zealand research programme, since the disease may result from the interplay between diet and genotype. Important components of innate immune responses are the Toll-like receptors (TLR) and mutations in the genes for TLR4, TLR9 and NOD2 (linked with TLR2) are associated with Crohn's disease. We have used specific TLR ligands to target an anti-inflammatory assay to these Crohn's associated TLR pathways. Common foods were subjected to organic solvent or water extraction and assayed for anti-inflammatory activity by their ability to suppress production of TNF α from a macrophage cell line. Foods showing high activity (e.g. apple, kiwifruit and avocado) were selected for assay using specific TLR4 (purified lipopolysaccharide), TLR 9 (bacterial CpG oligonucleotide), TLR 2 (purified gram positive peptidoglycan) and NOD2 (muramyl dipeptide) specific ligands. These extracts were then fractionated further to help to identify the pathway specific anti-inflammatory components of these foods. This preliminary screen allowed for the selection of foods ingredients that may be beneficial for people with Crohn's disease and other inflammatory gut conditions. Funded by the Foundation for Research, Science and Technology, New Zealand. C02X0403 FRST contract number.

THE EFFECT OF POLYPHENOLS AND OTHER FOOD COMPONENTS ON TNF- α - INDUCED INTESTINAL EPITHELIAL TIGHT JUNCTION PERMEABILITY

Dilip Ghosh¹, Aselle Adaim¹, Denis Lauren², Kevin Sutton³, and Margot Skinner¹

The Horticulture and Food Research Institute of New Zealand Ltd., Auckland¹, Ruakura², New Zealand

Crop and Food Research, Palmerston North³, New Zealand

Crohn's disease (CD) is associated with an abnormal increase in intestinal epithelial permeability and a defect in the intestinal tight junction (TJ) barrier has been proposed. Levels of the inflammatory mediator tumour necrosis factor- α (TNF- α) are markedly increased in CD patients, and as this mediator can increase intestinal TJ permeability, it may be a contributing factor in the intestinal permeability defects of CD. The objective of this study was to devise a food-based therapeutic approach to retighten the leaky TJ barrier. An *in vitro* model, consisting of filter-grown human intestinal epithelial Caco-2 monolayer was used to investigate the potential of polyphenols and other common foods on transepithelial electrical resistance (TEER), as a measure of TJ integrity. The effect on the functional localization of the TJ protein zonula occludens-1 (ZO-1) by the immunofluorescent antibody method was also determined. TNF- α (10-100 ng/ml) was shown to produce a concentration- and time-dependent (up to 72 h) increase in TJ permeability in this model. Two NF- κ B inhibitors, curcumin and triptolide, prevented the effect of TNF- α in a concentration- and time-dependent manner. Kiwifruit extracts prepared using solvent or water extraction methods were tested and some extracts were shown to inhibit the TNF- α induced TJ permeability. Progressive disturbances in the ZO-1 protein at cellular borders with a gap-like appearance were also noticed. These results demonstrate that some food-derived polyphenols have the ability to prevent TNF- α -induced intestinal epithelial tight junction permeability and foods or food components with this property may be useful for formulating personalized nutrition for CD and other intestinal disorders associated with an increase in intestinal epithelial permeability.

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Biomarkers of gut inflammation: Urine NMR

Grainger P^{1*}, Butts C^{2*}, Dommels Y^{2*}, Rowan D^{3*}, Roy NC^{4*}, Browning B^{5*}, Copp B⁶, Love D^{7*}, and Ferguson L^{1*}, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand¹, Crop and Food Research, Palmerston North, New Zealand², HortResearch, Palmerston North, New Zealand³, Metabolism & Microbial Genomics, Food & Health Group, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand⁴, Department of Nutrition⁵, Department of Chemistry⁶ and School of Biological Sciences⁷, University of Auckland, Auckland, New Zealand. Members of Nutrigenomics NZ*.

Crohn's Disease is an inflammatory bowel disease (IBD) characterised by inflammatory destruction of the intestinal wall. At present, methods for determining inflammation of the bowel are costly, time consuming and can cause discomfort to patients especially in the case of invasive endoscopy. The aims of this project are 1) to establish a non-invasive means of determining the Crohn's Disease state using a biomarker strategy and once this is established 2) to test the efficacy of various dietary interventions as a treatment paradigm for those at risk of this disease. Several non-invasive tissues which included blood, urine, faeces, and buccal swabs were obtained from a MDR1a knockout mouse model of IBD and from FVB control mice. Samples were examined by microarray, cytokine analysis and metabolomic profiling. The most promising results to date have been obtained by metabolic profiling of urine samples obtained from knockout and control mice using nuclear magnetic resonance (NMR) methods. Three methods of NMR were investigated; the phosphorous (³¹P) NMR spectra showed no difference between the two mouse types with only one very broad peak being observed; both the ¹³C and the proton (¹H) NMR spectra however showed marked differences in the intensities of the metabolites present in the urine of MDR1a knockout versus FVB control mice. The proton NMR spectrum presented a pair of doublets (5.1-5.2 ppm) that were more dominant in MDR1a mice urine compared to the controls. These doublets are pivotal as they will enable the identification of the two contributing compounds which are the most significant candidates for biomarkers so far identified through this research.

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Biomarker strategies for the analysis of Crohn's Disease

Grainger P¹, Roy NC², Love D³, and Ferguson L¹, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand¹, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand², School of Biological Sciences, University of Auckland, Auckland, New Zealand³.

Crohn's Disease (CD) pathogenesis is caused by an excessive immune response to environmental factors in genetically susceptible people. Environmental factors known to have an effect on the pathogenesis of CD are gut microflora and food components; however, the exact species of microflora or components of foods require further investigation to understand their role in disease pathogenesis. Several genetic factors have also been identified as contributors to disease, a high proportion of which are linked to the NF- κ B pathway and lead to the transcription of a large number of pro-inflammatory genes. Current methods of analysing CD are not pleasant for the patient and are costly and time consuming. In order to bridge this gap in our knowledge, there is a need to develop methods that allow rapid diagnosis and even prognosis using easily accessible tissues from CD patients. In order to address this need, blood, stool and urine samples taken from a mouse model of CD have been screened for changes in transcript, protein and metabolite profiles compared to non-diseased mice. The strengths and limitations of the methods that have been used and the tissues that have been accessed will be discussed with a view to identifying a practical biomarker strategy that can assist CD patients prior to disease onset as well as during disease progression.

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Grainger P^{1*}, Butts C^{2*}, Dommels Y^{2*}, Rowan D^{3*}, Roy NC^{4*}, Browning B^{5*}, Copp B⁶, Love D^{7*}, and Ferguson L^{1*}, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand¹, Crop and Food Research, Palmerston North, New Zealand², HortResearch, Palmerston North, New Zealand³, Metabolism & Microbial Genomics, Food & Health Group, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand⁴, Department of Nutrition⁵, Department of Chemistry⁶ and School of Biological Sciences⁷, University of Auckland, Auckland, New Zealand. Members of Nutrigenomics NZ*.

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Biomarker strategies for the analysis of Crohn's Disease

Grainger P¹, Roy NC², Love D³, and Ferguson L¹, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand¹, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand², School of Biological Sciences, University of Auckland, Auckland, New Zealand³.

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SUSCEPTIBILITY GENES FOR CROHN'S DISEASE IN NEW ZEALAND PATIENTS

J Hong¹, E Leung¹, T Merriman², A Fraser³, and GW Krissansen¹, Departments of Molecular Medicine & Pathology¹, and Medicine³, University of Auckland, Department of Biochemistry², University of Otago

Crohn's disease (CD) is a chronic inflammatory bowel disease affecting ~1 in 1000 people in western countries. The prevailing theory is that immune system in genetically susceptible individuals responds inappropriately to intestinal bacteria. To date, only the *CARD15* gene which encodes a pattern recognition receptor (PRR) that helps our immune system respond to bacteria, was found to be a significant risk factor for CD. CD is thought to be a multigene disease where the risk of developing CD depends on the number and type of susceptibility genes you inherit. Therefore, we aimed to identify gene polymorphisms that may increase the risk of CD. The frequency of 23 polymorphisms in a total of 15 candidate genes was examined in 182 CD patients and 188 controls by PCR-RFLP. The major *CARD15* polymorphism 3020insC was found to be associated with CD ($P < 0.0001$), and increases the risk of terminal ileum disease and need for surgery. An *OCTN1/2* TC haplotype (*SLC22A*-TC) was associated with CD ($P = 0.03$) and with an ileocolonic location. No other candidate polymorphisms were associated with CD. Only the damaged *CARD15* and *OCTN* genes were significant risk factors for New Zealand CD patients.

In addition, we found that *CARD15* generates an array of alternatively spliced RNA transcripts that potentially produce a variety of truncated *CARD15* proteins with different biological roles. When monocytes expressing *CARD15* were incubated for 5 h with H37Ra bacteria, *CARD15* transcripts were down-regulated. The alternative splicing of *CARD15* transcripts could be part of a complex mechanism to regulate intracellular sensing of bacteria.

All of the studies described were approved by the Auckland and Otago Ethics Committees. Supported by the Broad Medical Research Foundation (California), and the Auckland Medical Research Foundation.

EFFECT OF INOCULATION WITH INTESTINAL BACTERIA ON INTESTINAL GENE EXPRESSION IN THE INTERLEUKIN-10 KNOCKOUT MOUSE

MPG Barnett¹, ST Zhu^{2*}, A Cookson¹, R Broadhurst^{3*}, B Knoch^{1*}, WC McNabb^{1*} and NC Roy^{1*}.

¹Metabolism & Microbial Genomics Section, Food & Health Group, AgResearch Limited, Palmerston North, New Zealand, ² Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand, ³ National Resources Group, AgResearch Limited, Hamilton, New Zealand.

The interleukin-10 (IL-10) knockout (KO) mouse develops Crohn's disease-like colitis when raised under conventional conditions. This colitis is caused in part by an inappropriate response to normal intestinal bacteria. Our aim was to produce an improved model of inflammation by inoculating these mice with normal intestinal bacteria, and characterize gene expression changes resulting from inoculation. IL-10 KO and C57BL/6J (control) mice were orally inoculated with 12 *Enterococcus* strains (EF) and/or complex intestinal flora (CIF) collected from C57BL/6 mice raised under conventional conditions. At 12 weeks of age, intact intestinal sections were assigned a histological score (HIS) based on inflammatory cell infiltrates and tissue destruction. RNA extracted from intact colon was labelled with Cy3 dye and hybridized on Agilent 44k arrays with a Cy5-labelled reference RNA sample. The HIS (particularly colon) of IL-10 KO mice inoculated with EF + CIF was higher ($P < 0.05$) than that of control mice, and more consistent compared to IL-10 mice which were not inoculated. These results show that IL-10 KO mice inoculated with intestinal bacteria are an improved model of intestinal inflammation. The significance of gene expression changes resulting from bacterial inoculation will be discussed.

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THE EFFECT OF BOVINE COLOSTRUM SUPPLEMENTATION ON SALIVARY IgA IN RUNNERS

Christine V Crooks¹, Clare R Wall¹, Martin L Cross², Kay J Rutherford - Markwick¹
Institute Food Nutrition and Human Health¹, Massey University, Centre of Innovation²
University of Otago

Background: Secretory IgA in the saliva (s-IgA) is a potential immunological correlate of Upper Respiratory Tract Infection (URTI) status. Reduced s-IgA levels have been shown to precede the incidence of URTI in athletes. Nutritional supplements such as bovine colostrum may improve mucosal immunity and could be beneficial to the health of an athlete.

Purpose: The aim of this study was to measure levels of s-IgA in recreational distance runners to determine whether bovine colostrum has an effect.

Method: Thirty-five distance runners (15 female, 20 male, age 35-58 years old), consumed either bovine colostrum or placebo for 12 weeks. Saliva samples were taken prior to training at baseline, monthly and two weeks post supplementation. Daily training and wellness records were kept and seven day diet records were completed twice during the study.

Results: Median levels of s-IgA increased by 79% in the colostrum group after 12 weeks intervention, and the time-dependent change from baseline value was significant ($p = 0.0291$). This significance was still seen after adjusting for training volume and the reporting of upper respiratory symptoms (URS). Median levels of s-IgA also increased in the placebo group by 16% but this was insignificant compared to baseline levels.

Conclusion: This study has demonstrated an increase in levels of s-IgA among a cohort of athletes following colostrum supplementation. While this result is statistically significant, its physiological interpretation must be viewed with caution due to the small numbers in this study and the large variability in s-IgA levels measured.

The study described was approved by the Auckland Human Ethics Committee.

THE EFFECTS OF NATTO, A TRADITIONAL JAPANESE FOOD, ON METABOLIC SYNDROME

Tetsu Kinoshita¹, Yumi Itoh¹, Naomi Kaneko², Chiaki Honda², Youko Okumoto³, Fusayoshi Satoh³, Jiyoong Kim⁴, and Masafumi Kitakaze⁴

HuBit genomix, Inc¹, Life Science Business Unit², NTT DATA Corporation, Department of Health and Welfare³, Arita-Town Office, Cardiovascular Division of Medicine⁴, National Cardiovascular Center

Natto is a traditional Japanese food that is made of fermented soybeans by *Bacillus natto*. Natto is known as one of health promoting foods due to its rich source of active components, such as isoflavone, vitamin K2, and the enzyme of natto-kinase. Natto-kinase has a potential to break down fibrin thrombi *in vitro*. However, no clinical studies using Natto have been held to confirm the health benefiting claims. In this pilot study, we attempted to develop study methods to verify the preventive effects of Natto against metabolic syndrome, which is a typical lifestyle-related disease and is a risk factor for cerebrovascular and cardiovascular diseases.

61 residents in Arita-Town, Saga Prefecture were enrolled in the study. These subjects had at least one warning signs in their health profiles of obesity, blood pressure, serum glucose, and serum lipid levels, from their previous annual medical check-up. All subjects were asked to keep a dietary questionnaire through out the 4 weeks study. 52 subjects consumed additional 30g of Natto every day, while the 9 controls were asked to refrain from taking Natto. After 4 weeks, all of the participants were checked for the same clinical parameters plus adiponection level and comparisons were done. The results are presented and discussed with regard to appropriate study design to evaluate the effects of functional foods and to establish the primary protection model against lifestyle-related diseases.

This intervention study was approved by the Ethics Committees of Related Organizations.

MULTIPLEXED MICROSPHERE-BASED FLOW CYTOMETRIC IMMUNOASSAYS FOR SIMULTANEOUS DETECTION OF CYTOKINES IN SAMPLES FROM ANIMAL OR HUMAN CLINICAL TRIALS AND CULTURE SUPERNATANTS

Jingli Zhang, Aselle Adaim and Margot Skinner
HortResearch

ELISA is the traditional method used to quantify soluble analytes in biological samples and can only measure one analyte at a time. The multiplexed microsphere-based assays represent an innovative flow-cytometric approach, which can measure multiple analytes simultaneously in a small sample volume (<25 µL). Here, we described a multiplex fluorescent bead immunoassay (MFBI) for quantitative detection by flow cytometer of human or mouse cytokines in plasma, serum, cell-cultured supernatants or other body fluids.

The MFBI technology used a flow cytometer to distinguish two sets of beads of different size and fluorescent intensities. Each set consists of five bead populations internally dyed with varying intensities of a fluorescent dye. Thus ten cytokines can be simultaneously quantified in the same sample. A mixture of coated-beads for each cytokine was incubated with the samples or standard mixture. Cytokines in the sample bind to the antibodies linked to the coated-beads. A biotin-conjugated second antibody mixture was added, these antibodies bond the cytokines captured by the first antibodies. Then, streptavidin-PE was added and quantified by fluorescence, which corresponding to cytokine concentrations.

In this study, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α and IL-12p70 in human plasma, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, TNF-α and GM-CSF in mouse plasma and IL-1β, IL-6, IL-8, IL-10, THF-β, and IL-12p70 in cultured-cell supernatants were tested. Low reagent volumes, reduced incubation times and fewer washing steps make MFBI technique more cost-effective and efficient. Here we have demonstrated the feasibility of multiplexed cytokine detection in human, mouse plasma and cultured-cell supernatants and it can be expanded to detect other proteins such as adhesion molecules, caspases and signalling molecules.

This study was funded by the Foundation for Research, Science and Technology NSOF through HortResearch.

METABOLOMICS: APPLICATIONS IN NUTRIGENOMICS

DD Rowan, HortResearch, Private Bag 11 030, Palmerston North, New Zealand. Member of Nutrigenomics NZ*.

Metabolomics aims for comprehensive knowledge of the biochemicals, the “metabolome”, which characterises a particular organism. The composition of the metabolome (or metabolic profile) is influenced both by external factors such as diet and by the expression of the individual genome of each individual.

Characterisation and understanding of the metabolic profile allows definition of the health status of individuals. Similarly the health effects of foods might be understood in terms of their metabolic profiles, which may contain a vast array of phytochemicals occurring at widely differing concentrations. Little is as yet known of the extent to which changes in the diet elicit changes in metabolic profiles¹.

The application of the major analytical methodologies of metabolomics (NMR, GC- and LC-MS, and DI-ESI-MS) to nutritional genomics will be discussed using examples from the analysis of fruit and of urine and serum by us and by others.

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* Nutrigenomics New Zealand (www.nutrigenomics.co.nz) is a collaboration between AgResearch Limited, Crop & Food Research, HortResearch and The University of Auckland and is largely funded by the Foundation of Research, Science and Technology.

Se, SNPs and susceptibility to disease - do some people need more selenium?

John Hesketh, Human Nutrition Research Centre and Institute for Cell and Molecular Biosciences,
University of Newcastle, Newcastle-upon-Tyne, UK.

Selenium (Se) is essential for human health: sub-optimal intake increases risk of cancers and supplementation has been reported to reduce mortality from cancer of colon and prostate. The mechanisms by which Se intake modulates cancer risk are unknown.

The response of selenoprotein expression to low Se supply differs between selenoproteins and between tissues. The 3' untranslated region (3'UTR) of the selenoprotein mRNAs play a role in this prioritisation of Se. In Caco-2 cells and rat colon expression of selenoprotein W was found to be sensitive to Se supply. In humans microarray analysis of leukocyte gene expression is being carried out to identify downstream targets of Se supplementation

Potentially, SNPs in either selenoprotein genes involved in Se transport or in selenoprotein gene 3'UTRs could influence Se metabolism and affect the response of individuals to selenium supplementation. A common SNP has been identified at position 718 in the 3'UTR of the GPX4 gene. The functional significance of this SNP is being determined by reporter gene studies, supplementation trials in which individuals are supplemented with 100ug/day sodium selenite for 6 weeks and by disease association studies with patients suffering from ulcerative colitis and colon cancer. In addition we have screened cohorts of Caucasians, Chinese and South Asians for SNPs in the selenoprotein P and selenophosphate synthetase 2 genes. Gene regions with potential polymorphisms were identified by DNA-HPLC and SNPs confirmed by sequencing. The influence of two SNPs in the selenoprotein P gene on biomarkers of Se status is being determined in a Se supplementation trial.

Supported by the UK Food Standard Agency, BBSRC, World Cancer Research Fund and European Nutrigenomics Organisation (contract FP6-506360).

EPIGENETIC CONSEQUENCES OF NUTRITIONAL DEFICIENCY IN PREGNANCY

M Dziadek¹, S Ravelich¹, G Konycheva¹, G Smith¹, S Patel¹, M Vickers², B Breier² and M Ehrich³, School of Biological Sciences¹, and Liggins Institute², University of Auckland, and Sequenom Inc³, San Diego, USA

Epigenetic modifications of the genome are implicated in phenotypic differences in physiological fitness and disease susceptibility, and could account for the increasingly recognized links between the prenatal and early postnatal nutritional environment and adult health and disease. A mismatch between nutrient availability in early and later life appears to precipitate the emergence of major adult and increasingly childhood onset diseases: obesity, cardiovascular disease, type 2 diabetes and cancer. Epigenetic silencing of imprinted genes through DNA methylation and histone modification is an important mechanism underlying growth and development. We have used a rat model to investigate whether methylation and allelic expression of the *Igf2* and *H19* genes are influenced by maternal undernutrition during pregnancy.

We demonstrate altered levels of methylation of the differentially methylated regions of the *Igf2* gene in fetal tissues from undernourished offspring when compared to those on an *ad libitum* control diet, and these differences are maintained into adulthood. These changes are associated with tissue-specific reactivation of the silenced maternal *Igf2* allele, indicating loss-of-imprinting (LOI), which only becomes evident after birth. LOI of *Igf2* does not, however, cause an increase in *Igf2* expression at a tissue level. Our studies indicate that maternal nutrition does alter the epigenetic state of the offspring genome by affecting molecular mechanisms underlying imprinting. Such epigenetic alterations in early life may contribute to epigenetic variability within the population that influences the risk of diseases in adulthood.

All of the studies described were approved by the University of Auckland Animal Ethics Committee. Supported by the Marsden Fund.

Nutrition, Epigenetics and Disease Susceptibility

Randy L. Jirtle, Ph.D.

Duke University Medical Center, Durham, NC 27710 USA

Human epidemiologic and animal data indicate that susceptibility to adult-onset chronic conditions such as cardiovascular disease, diabetes, obesity, and cancer is influenced by persistent adaptations to prenatal and early postnatal nutrition [1]. We have used the viable yellow agouti (A^{vy}) mouse, which harbors a retrotransposon upstream of the Agouti gene, to investigate the importance of maternal nutrition and DNA methylation in determining the susceptibility of offspring to adult diseases. We have shown that maternal dietary supplementation during pregnancy with either methyl donating substances (i.e. folic acid, vitamin B₁₂, choline and betaine) [2] or genistein [3], a phytoestrogen present in soya products, alters coat color of the offspring by increasing CpG methylation of the transposable element upstream of the Agouti gene rather than by gene mutation. Furthermore, these epigenetic changes reduce the risk of developing obesity in the offspring - a clear example of nature via nurture. It is now critically important to determine if similar epigenetically labile genes exist in the human genome, and whether micronutrients are useful in counteracting environmentally-induced deleterious alterations of the epigenome. (Supported by NIH grants CA25951, ES08823 and ES08823)

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PROTEOMICS OF ACTIONS OF GRAPE SEED EXTRACT IN THE BRAIN; EXTRAPOLATION TO THE GUT.

• H Kim^{1,2,3}, J Deshane¹, P Hall¹, P Sarkar¹, S Meleth^{3,4}, S Barnes^{1,2,3}.

Department of Pharmacology and Toxicology¹, UAB Comprehensive Cancer Center Proteomics/Mass Spectrometry Shared Facility², UAB Center for Nutrient-Gene Interaction in Cancer Prevention³, Department of Medicine-Division of Biostatistics⁴, University of Alabama at Birmingham, Birmingham, AL 35294 USA

Grape seed extract (GSE) has been suggested to have health benefits in part due to the anti-oxidant activity of its polyphenols; we hypothesized that part of the previously shown neuroprotective activity of GSE involved protection against protein oxidations. We utilized two-dimensional electrophoresis (2DE) and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and LC-tandem MS (LC-MS/MS) to assess differences in brain proteins in a mouse model of dementia (Hsiao et al., 1996) following administration of diet containing 5% GSE. Image and statistical analysis of 2D gels indicated that several brain proteins were different in abundance or isoform complexity between GSE versus CT brains. To determine effects on protein oxidations, brain protein carbonyls were derivatized with dinitrophenylhydrazine creating a DNP moiety, then subjected to 2DE Western blot analysis with anti-DNP antibody. GSE appeared to induce a global reduction in protein oxidations, including on tubulin, the microtubule subunit. Increased oxidation of tubulin was shown to be a marker for inflammatory bowel disease (IBD). Thus GSE might be a basis for treatment against IBD by protecting against oxidation of critical proteins such as tubulin.

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NUTRIENT-GENE INTERACTIONS IN MODULATING IMMUNE RESPONSE

MA Skinner¹, D Forbes¹, D Lauren¹, K Sutton² and L MacKay³ and M Philpott³ HortResearch¹, New Zealand Institute for Crop and Food Research Ltd,² School of Medical and Health Sciences, University of Auckland,³ New Zealand email mskinner@hortresearch.co.nz

Innate immunity is the rapid and generalized inflammatory response that is the first organised reaction to infection. It involves the recognition of a limited number of pathogen associated molecules by Toll-like receptors (TLR) leading to the production of a complex variety of soluble mediators, such as interleukin - 1 (IL-1) and tumour necrosis factor α (TNF- α). Adaptive immunity is highly specific for a particular pathogen and like the innate response is directed by cytokines. Dysregulated inflammation leads to classic inflammatory diseases such as the inflammatory bowel diseases and other chronic conditions including cardiovascular disease. Nutrients, for example, fish oil and phytochemicals present in fruit, tea and spices, can modulate genes involved in regulating immune responses. Nutrient effects on inflammatory mediators have been shown to exhibit inter-individual variation based on specific single nucleotide polymorphisms (SNPs). Thus immune response genes are good targets for modulation by nutritional compounds. An objective of the Nutrigenomics NZ programme is to discover activities in foods that might rectify dysregulated inflammatory immune responses in patients with Crohn's disease. To screen for activity inflammatory pathways involving TLR4, TLR9 and NOD2 have been targeted, as some Crohn's disease patients have SNPs in these receptors and a gene-nutrient sensor assay for NOD2 has been developed. Results from different types of common food extracts will be presented and those that that might modify gut mucosal immune responses discussed.

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MODELLING THE GASTROINTESTINAL SYSTEM

AJ Pullan, Bioengineering Institute, University of Auckland

Between sixty and seventy million people in the US alone are affected by gastrointestinal disorders. Many of these conditions are consequences of abnormal electrophysiological behaviour but are difficult to assess without surgical intervention. Thus, there is a need to develop accurate non-invasive techniques to aid in the clinical assessments of gastrointestinal disorders. It has recently been shown that it is possible to record the magnetic fields corresponding to the muscular activity within the stomach and intestine using specialist recording devices. However, the interpretation of these magnetic recordings remains a challenge. In particular, a greater understanding of the relationship between cellular level activity and the resultant external magnetic fields is needed. To that end, we have begun developing extensible anatomically and biophysically-based models of the human gastrointestinal tract.

In this talk the latest results of this modelling work will be presented. In particular, movies, showing how the model has been constructed, and how the stomach and intestine behave electrically, will be shown. We will also show how we can simulate some abnormal events that are associated with certain disease states.

Whilst the original modelling framework was developed to help understand electrical activity of the gastrointestinal system, it has subsequently begun to find many other uses (for instance, as an aid in helping understand the gastro-oesophageal junction). The use of the modelling work in these new application areas will also be presented.

A/Prof Andrew Pullan

Department of Engineering Science and Bioengineering Institute

The University of Auckland, Private Bag 92019

Auckland, New Zealand

Ph: (649) 373 7599 x88399, Fax: (649) 367 7157

www.esc.auckland.ac.nz/Pullan

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Intestinal epithelial cell signaling and host-derived negative regulators under chronic inflammation: from the proteome to specific mechanisms.

Prof. Dr. Dirk Haller; Technical University of Munich, Else Kroener-Fresenius-Center for Experimental Nutritional Medicine, Am Forum 5, Freising-Weihenstephan, Email: haller@wzw.tum.de

It has become clear from numerous studies that enteric bacteria are a critical component in the initiation and perpetuation of chronic intestinal inflammation. An emerging new paradigm suggests that the lack of host-derived control mechanisms may lead to functional and immune disturbances at the intestinal epithelial cell (IEC) level. We use *Enterococcus faecalis* as a model organism to study the initiation and perpetuation of experimental colitis in Interleukin 10 gene deficient mice (IL-10^{-/-}). We showed that *E. faecalis* transiently induced toll-like receptor (TLR) 2-mediated RelA phosphorylation and NF- κ B-dependent gene expression in native IEC from wild type mice but persistent activation of the TLR/NF- κ B pathway in chronically inflamed IL-10^{-/-} mice. Interestingly, the induction of nuclear NF- κ B RelA phosphorylation at early stages of bacterial colonization in the germfree host was associated with the activation of protective TGF- β -mediated Smad2 signaling in wild type but not IL-10^{-/-} IEC. Mechanistically, transforming growth factor (TGF)- β -activated Smad signaling induced TLR2 protein degradation (Ruiz et al. J. Immunol. 2005) and inhibited CBP/p300-mediated histone phosphorylation in IEC (Haller et al. J. Biol. Chem. 2003). To identify additional disease mechanisms for the induction of experimental colitis, we used functional epithelial cell proteomics in *E. faecalis*-monoassociated wild type and IL-10^{-/-} mice. Interestingly, the persistent induction of NF- κ B RelA phosphorylation in IL-10^{-/-} IEC was associated with the increased expression of the glucose regulated protein (grp)-78. Mechanistically, we showed that grp-78 recruitment to the IKK complex was required for TNF-induced NF- κ B RelA phosphorylation, suggesting pro-inflammatory mechanisms for grp-78 expression in IEC. Important for the molecular understanding of host-derived regulation, IL-10 blocked TNF-induced RelA phosphorylation in IL-10 receptor reconstituted IEC and IL-10-mediated p38 signaling was present in wild type but not IL-10^{-/-} IEC. It seems an attractive hypothesis that host-derived feed-back mechanisms control the status of epithelial cell activation (Haller. Neurogastroenterol. Motil. 2006). Similar to the findings for TGF- β , IL-10 signaling may critically contribute to immune homeostasis and the balance towards commensal enteric bacteria at the epithelial cell level through the regulation of the endoplasmic reticulum (ER) stress response mediator grp-78. In addition to grp-78 ER stress responses, changes in creatine kinase, serpin and galecin-3 expression affected NF- κ B signalling, energy homeostasis and apoptosis/barrier function in IEC under conditions of chronic inflammation.

THE GUT MICROBIOTA IN HEALTH AND DISEASE

GW Tannock, Department of Microbiology and Immunology, University of Otago

Complex microbial communities, in which bacteria predominate, inhabit distal regions of the human digestive tract. Perhaps as much as 50% of the bacterial types comprising these bacterial communities has not yet been cultivated by traditional bacteriological methods. These uncultivated bacterial types are novel and are known only by their 16S ribosomal RNA gene sequences. Investigation of bowel communities therefore requires the use of nucleic acid-based analytical methods. Beneficially, bacterial communities in the bowel are likely to impact on the physiological and immunological development of the human host, especially during the first few months of life. Adversely, it seems that the bacterial communities act as surrogate pathogens in inflammatory bowel diseases (Crohn's disease and ulcerative colitis). The manipulation of the composition or metabolic activities of the bacterial communities therefore offers potential prophylactic or therapeutic strategies for specific human diseases. Modification of bowel community composition and function may best be obtained by dietary manipulation, and undigested complex carbohydrates delivered to the large bowel might accomplish such changes. Research results from the investigation of bowel bacteria in relation to the activation of neonatal immune cells, the composition of the stool of normal and inflamed pelvic pouches, and the effect of feeding rats a diet supplemented with preparations of high viscosity beta-glucan, will be used to illustrate these topics.

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The human and experimental animal studies described were approved by ethical committees in Canada and New Zealand. Supported by Alberta Agriculture, Alberta Value Added Corporation, Child Health Foundation, Crohns and Colitis Foundation of America, and the University of Otago.

RECENT ADVANCES IN MODELLING OF HUMAN DIGESTIVE PROCESSES

MSJ Wickham¹ and RM Faulks¹, Model Gut Exploitation Platform¹, Institute of Food Research,
Colney Lane, Norwich NR4 7UA, UK

Ten years of pioneering research on the physiology of the human gut is being developed into a revolutionary new research tool that will enable the food and pharmaceutical researcher to predict digestion of real foods and medicines within the human gut. At the Institute of Food Research, UK, (IFR) a “state of the art” *in vitro* system that simulates the human stomach and small intestines, for the first time from a true physiological perspective, is being constructed. As well as providing an alternative to some animal experiments and human studies, the IFR Model will shed light on important areas such as the fate of nutrients and medicines taken orally, and interactions between foods and medicines.

The IFR Model of human digestion is the first model developed to combine new and emerging scientific knowledge of the physical, mechanical, and biochemical environments experienced by the luminal contents, and therefore provides a true simulation of the human stomach and small intestine. This means, for example, that information about digestive enzymes can be integrated with the mechanical forces experienced within the gut to increase our understanding of the effects of different food and pharmaceutical structures and compositions on overall digestion. The IFR Model therefore provides realistic and predictive simulations based on up to date knowledge of the digestive processes. It can handle real foods and pharmaceutical preparations and allows access at any stage of ‘digestion’, permitting sample collection and analysis at any time point.

Biochemical and molecular characterization of Bifidobacterial bile salt hydrolases and their applications.

Byong H. Lee ^{1,2*} and Guen Bae Kim¹

¹McGill University, Dept. of Food Science and Agricultural Chemistry and ²AAFC Food R & D Centre, Montreal, QC, Canada H9X 3V9

Bile salt deconjugation is the most biologically significant reaction among the bacterial alterations of bile acids in the GI tract of human and animal. Bile salt hydrolase (BSH, EC 3.5.1.24), which catalyzes the hydrolysis of glycine- and/or taurine-conjugated bile salts into amino acid residues and free bile acids in the intestine is widely distributed in many GI microbes and bacterial groups that have been commonly used for probiotics. Knowledge gained through BSH research will provide further insight into the physiological impact, the survival of probiotics and the hypocholesterolemic effect. In spite of this wide distribution and high activity of BSH enzyme in bifidobacteria, little information is available on the BSH at the biochemical and molecular levels. Most of strains in the genus *Bifidobacterium* showed high BSH enzyme activity and some type specific characteristics were also observed. To investigate the molecular characteristics of the gene encoding bile salt hydrolase, three *bsh* genes from different species were cloned, sequenced and the upstream/downstream of this gene was characterized. The *bsh* genes were also very useful for the identification and phylogenetic analysis of *Bifidobacterium* species.

Title: Nutrigenomics approach to the management of inflammatory diseases

Author: Slavik Dushenkov, Julie B Hirsch, and David A Evans

Affiliation: WellGen, Inc. New Brunswick, NJ, USA

Effect of bioactive compounds from food on gene expression is widely recognized. In the last several years an increasing body of scientific evidence has demonstrated that ability of individual compounds, as well as complex mixtures of chemicals, derived from food alter the expression of proinflammatory genes in the humans. Several human diseases result in multiple inflammatory responses which are associated with many diseases including arthritis, cancer, cardiovascular disease, dermatitis, asthma, obesity, and others. Detailed mechanisms of action as to how food derived components play an active role in prevention of inflammation have been elucidated. Such biologically active compounds include theaflavins and catechins from tea, curcumin from turmeric, resveratrol from grapes, and chicory. While chronic diseases are very complex, an opportunity exists to regulate genes involved in inflammation by enriching our diet with the specific foods inherently rich in such compounds, enriched foods containing standardized extracts of well studied sources, or dietary supplements. We developed a vertically integrated approach for development of nutrigenomics products for treatment and prevention of inflammation. Black tea based dietary supplement developed through this process is being tested in humans.

Zinc fluxes and zinc transporter genes in chronic diseases

Chiara Murgia , Chiara Devirgiliis, Peter Zalewski* and Giuditta Perozzi

**INRAN, National Research Institute on Food and Nutrition, Via Ardeatina 546,
00178 Roma, Italy**

***Department of Medicine, University of Adelaide, The Queen Elizabeth
Hospital, Woodville, South Australia**

The group IIb metal zinc (Zn) is an essential dietary component that can be found in protein rich food such as meat, sea food and legumes. Oysters are particularly rich in this metal. Zinc regulates a number of cellular processes including mitosis, apoptosis, secretion and signal transduction. Imbalance in zinc homeostasis was found to be correlated to a number of chronic diseases such as asthma and diabetes. Zinc ions cross biological membranes with the aid of specialized membrane proteins, belonging to the ZIP and ZnT families. The ZIPs are coded by the Slc39a gene family and are responsible for uptake of the metal, ZnTs are coded by the Slc30a family and are involved in intracellular traffic and/or excretion. Both the ZnTs and Zips exhibit unique tissue-specific expression, differential responsiveness to dietary zinc deficiency and excess, as well as to physiologic stimuli via hormones and cytokine. Serum Zn concentrations are significantly lower in patients with Type I diabetes than in healthy controls and dietary zinc supplementation protects mice against chemically induced diabetes. Zn is involved in insulin synthesis, storage and secretion in the pancreatic β cells in the islets of Langerhans. A total of 16 ZIP and ZnT transporters are expressed in the pancreas and regulate zinc fluxes. RT-PCR and immuno-staining with a specific antibody, show that the ZnT8 gene is expressed specifically in islets cells. We studied the features of the corresponding protein and its intracellular localization in β -TC6 cells, which secrete insulin in response to glucose. We show that zinc depletion interferes with glucose stimulation of insulin secretion, pointing to Zn and Zn transporter genes as important players in the management of diabetes.

Introduction to nutrigenomics for the food industry

Lynnette R. Ferguson,

Discipline of Nutrition, The University of Auckland, Private Bag 92019,

Auckland, New Zealand

It has been suggested that the supermarket of today will be the pharmacy of tomorrow. Such statements have been derived from recognition of our increasing ability to optimize nutrition, and maintain a state of good health through longer periods of life. The new field of nutrigenomics, which focuses on the interaction between bioactive dietary components and the genome, recognizes that current nutritional guidelines and recommended food choices may be ideal for only a relatively small proportion of the population. There is good evidence that nutrition has significant influences on the expression of genes, and, likewise, genetic variation can have a significant effect on food intake, metabolic response to food, individual nutrient requirements, food safety, and the efficacy of disease-protective dietary factors. For example, a significant number of human studies in various areas are increasing the evidence for interactions between single nucleotide polymorphisms (SNPs) in various genes and the metabolic response to diet, including the risk of obesity. Many of the same genetic polymorphisms and dietary patterns that influence obesity or cardiovascular disease also affect cancer, since overweight individuals are at increased risk of cancer development. The control of food intake is profoundly affected by polymorphisms either in genes encoding taste receptors, or in genes encoding a number of peripheral signaling peptides such as insulin, leptin, ghrelin, cholecystokinin, and corresponding receptors. Total dietary intake, and the satiety value of various foods, will profoundly influence the effects of these genes. Identifying key SNPs that are likely to influence the health of an individual provides an approach to understanding and, ultimately, to optimizing nutrition at the

population or individual level. Traditional methods for identification of SNPs may involve consideration of individual variants, using methodologies such as restriction fragment length polymorphisms or quantitative real-time PCR assays. New developments allow identification of up to 500 000 SNPs in an individual, and with

increasingly lowered pricings, these developments may explode the population-level potential for dietary optimization and development of novel foods based on nutrigenomic approaches.

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**FOOD TECHNOLOGY:
PROSPECTS AND LIMITATIONS IN DEVELOPING PERSONALISED FOODS**

KH Sutton, Food and Biomaterials Innovation Team, NZ Institute for Crop & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand.

Functional foods have been identified as a key trend in the food industry for some time. Demographic and social trends, along with an increasing body of scientific knowledge concerning food components and their physiological effects, have moved foods from a position of supplying simply basic nutrition to one where specialised foods are seen (by consumers and marketers) to protect against and (perhaps) prevent disease. The initial focus of the Nutrigenomics New Zealand research programme is the inflammatory bowel condition Crohn's disease, a condition that may have both genetic and environmental contributions. From a food science perspective, two primary food outcomes are likely from the research programme: 1) public good information regarding good and bad foods for people with specific genetic profiles will likely become available and 2) intellectual property in the form of designed nutrigenomic foods are likely to emerge. Our studies into foods influencing Crohn's disease have indicated that there may be certain foods or food fractions that could be active in reducing inflammation in this debilitating condition. How these materials might be integrated into hypothetical, personalised "Crohn's positive" foods will be discussed, keeping in mind the difficulty of formulating specific types of food products with desired ratios of carbohydrate, protein and lipids, while maintaining textural properties or the need to mask adverse sensory properties from a desirable but unpalatable food fraction. An example from another health end-point oriented research project will be used to demonstrate how some ideas, both preconceived and from the results of research, can not be adopted due to these practical limitations.

Use of biomarkers to validate the efficacy of foods

Grainger P¹, Roy N², Love D³, and Ferguson L¹

Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand¹,
AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand², Faculty of
Biological Sciences, University of Auckland, Auckland, New Zealand³.

Crohn's disease pathogenesis is caused by an excessive immune response to environmental factors in genetically susceptible people. Environmental factors known to have an effect on the pathogenesis of Crohn's disease (CD) are gut microflora and food components; however, the exact species of microflora or components of foods require further investigation to understand their role in disease pathogenesis. Several genetic factors have also been identified as contributors to disease, a high proportion of which are linked to the NF- κ B pathway and lead to the transcription of a large number of pro-inflammatory genes.

Current methods of analysing Crohn's disease are not pleasant for the patient and are costly and time consuming. In order to bridge this gap in our knowledge, there is a need to develop methods that allow rapid diagnosis and even prognosis using easily accessible tissues from CD patients. To address this need, blood, stool and urine samples from a mouse model of CD have been screened for changes in transcript, protein and metabolite profiles compared to non-diseased mice. The strengths and limitations of the methods that have been used and the tissues that have been accessed will be discussed with a view to identifying a practical biomarker strategy that can assist CD patients prior to disease onset as well as during disease progression.

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MASS CUSTOMISATION OF FOOD

Mike Boland, Fonterra Co-operative Group Ltd., Private Bag 11-029, Palmerston North, New Zealand.

The rapidly-developing field of Nutrigenomics leads naturally to the need for “personalised nutrition”, i.e. food that is designed to take into account a person’s individual genetic nutritional needs as well as their situational nutritional needs. The challenge for the food industry in the 21st century is to be able to meet all individual needs, while at the same time continuing to use mass-scale manufacturing to produce food economically.

Mass customisation is a term that refers to production of units of a manufactured product that have been assembled from a selected range of mass-produced prefabricated parts, giving the customer a wide choice, based on the large multiplicity of combinations that can be obtained from a relatively modest range of components. An important aspect of mass customisation is the involvement of the final customer in the design of the product prior to manufacture. A good example of this is how Dell operates.

Application of the mass customisation approach to food has its own special needs and challenges. The POSIFoods™ project is an early attempt, using the principles of mass customisation, to go further and produce a unique personalised nutrition product for each consumer. This is based on a combination of smart food ingredients, point of sale assembly and a smart customer interface. An outline of the system will be described.

Nutrigenomics and the Food Industry

Jim Kaput, PhD

NutraGenomics

The science of nutrigenomics is being applied first to healthcare for the diagnosis and treatments of chronic diseases and to personal nutrition for maintaining health and preventing chronic diseases. The common factors in these different applications are genetic testing to assess needs and the matching of genetic makeup to foods or food supplements. While the promise of nutritional genomics has been well accepted by many scientists and business people, converting the science to medical and food products and consumers remains a significant challenge. While individual genes and their variants have been linked to specific foods or nutrients, an individual dietary chemical may interact with many other gene products in the course of absorption, transport, and metabolism. Gene – gene and gene – environment interactions that alter biological processes may change gene variant – nutrient associations differently among individuals of different ancestral backgrounds. As importantly, many nutrigenomic and nutrigenetic experiments are not yet testing dose: how much of a nutrient is needed for a biological effect in an individual. While these challenges may seem significant, high throughput ‘omics’ technologies applied to the appropriate biological experiments, promises to unravel the web of genome – nutrient interactions in each individual.

CONSUMER PERSPECTIVE: EXPERIENCE OF A COMPANY SPECIALISING IN NUTRIGENOMICS

RD Gill-Garrison, Chief Science Officer, Sciona, Inc., 1401 Walnut St., Suite 203, Boulder, Colorado 80302 USA

Public interest in nutrigenomics has grown incredibly over the past 12 months with an increasing amount of coverage in the popular press, both print media and television helping to raise awareness of developments in the field. An obvious application for personalized nutrition include a focus on effective weight management tools for the prevention of obesity, which has reached epidemic proportions; as well as heart disease, which is still a leading killer in the Western world; both health conditions have risk profiles modifiable by dietary and lifestyle choices. Current knowledge of gene-diet interactions indicate credible, actionable advice that can be given to an individual at the present time to reduce elements of risk associated with factors such as cholesterol metabolism and folate metabolism. Sciona has been providing advice to consumers focused on particular nutrients for the past four years and has learned valuable lessons on how to communicate information to the individual as well as how this type of information can lead to greater compliance on particular dietary recommendations. Challenges to the emerging –omic applications of personalized nutrition, include the protection of the privacy and security of an individual’s information, ethical review of all aspects of emerging commercial applications and the development of clear regulatory guidelines. A further challenge involves the actual interface with the public -- consumers consistently report an expectation that genetics will play an increasing role in their daily lives. Personalization of information will require greater sophistication and education of the consumer, but also of the various stakeholders in the consumer’s health care and nutritional world – from the doctor to the pharmacist, to perhaps the grocery store dietitian, as the personalization takes on potential forms of the daily diet regime, the right biomarker tests to order, the functional food – even the personalized food products to choose off the shelf.

TITLE OF ABSTRACT TO BE PRESENTED AT THE MEETING

Title: **Nutrigenomics and the Food Regulatory Environment**

Author: **Dean Stockwell, General Manager**
 Food Standards Australia New Zealand (FSANZ)

Abstract:

Is our world ready for nutrigenomics and the food innovations that must surely arise from local and international research efforts? And even if consumers are ready – will the regulatory environment welcome your innovation.

Successful innovation has many components: an idea, an investor, a product, a customer need but these alone may not result in a successful outcome. For the greater the innovation, the more likely it is to be novel and the greater the novelty, the greater the regulatory challenges. These are hurdles the innovator and entrepreneur may expect, but until they are faced the nature and significance of these challenges may not be well understood. This paper considers some of the challenges that may arise and discusses possible approaches to enable innovative food products to come to market.

CROHN'S DISEASE - clinical aspects

Alan G Fraser, Department of Medicine, University of Auckland

Ulcerative colitis and Crohn's disease are chronic inflammatory diseases of the gastrointestinal tract. They are identified by characteristic endoscopic/radiological and histological appearances. Ulcerative colitis and Crohn's disease have many clinical features in common but are best considered as separate disease entities rather two ends of a spectrum. Smoking increases risk of Crohn's disease but decreases risk of ulcerative colitis.

Crohn's disease may affect any part of the gastrointestinal tract. It is typically discontinuous, begins as "aphthous" ulcers within the epithelium overlying lymphoid aggregates, then ulceration becomes fissuring or deeply penetrating. There is transmural inflammation (affecting mucosa, submucosa, muscle coat and serosa) and the most specific feature are small, non-caseating epitheloid granulomas.

Symptoms depend on area of bowel involved. Small bowel - abdominal pain, nausea, diarrhoea, weight loss. Terminal ileal disease - pain and localised tenderness in right iliac fossa. Colonic disease - diarrhoea with blood. Perianal disease - abscess and fistulae - presents with pain and discharge.

Two main types of pain with small bowel Crohn's disease

1. Sub-acute obstruction - mid-abdominal cramping pain; onset 30-60mins after a meal - may not resolve with medical treatment.
2. Inflammatory pain - localised to right iliac fossa; more constant pain – unaffected by meals, aggravated by movement. Correlates more with inflammatory activity and resolves with medical treatment

Complications

1. intestinal obstruction - subacute
2. fistulae - from bowel to bowel, bladder, vagina, abdominal wall
3. nutritional deficiencies - anaemia, hypoproteinaemia , malabsorption

Medical management - nutritional deficiencies need to identified and treated.

Supplemental polymeric liquid diet may be helpful particularly to enhance growth in children. Steroids and 5-ASA containing drugs - benefit in active disease. Immunosuppressives (azathioprine) - steroid-sparing agents. Surgery indicated because of failed medical treatment or complications. Resection of diseased segment of bowel relieves symptoms but does not cure disease. Symptomatic recurrence after surgery 50% at 5 years.

DIETARY FACTORS AND GENOMIC STABILITY – THE LINK.

Graeme P Young, Flinders Cancer Control Alliance, Flinders University, Adelaide South Australia

Cancer results from a disordered and unstable genome. Instability might arise as a result of chance events or mistakes especially at the time of DNA replication. Alternatively, they might be caused by an environmental carcinogen where an adduct forms from interaction of carcinogen with DNA base or by irradiation. The consequence depends in part on whether the cell can act to “repair” the damage. If repair is effective, it would abort any downstream consequences while an unrepaired and thus mutated cell might develop into a pro-oncogenic clone.

In an effort to “repair” damage, two main events can occur. To effect repair of a DNA base, cell cycle arrest is triggered and repair enzymes restore a normal gene. The alternative is destruction of the DNA-damaged cell through activation of programmed cell death (apoptosis). If either fails, a viable cell that carries a pathogenic mutation might survive.

Dietary factors might interact directly with the genome (as a genetic or epigenetic regulator) or indirectly by influencing “repair” responses and so stabilise DNA.

A range are proapoptotic in vitro including fermentative production of butyrate. When dietary fibre is fed to rodents, active colonic fermentation generates high levels of butyrate which in turn are associated with an augmentation of the colonic apoptotic response to carcinogen and protection against cancer. Other studies show that a nonsteroidal antiinflammatory agent restores a defective apoptotic response to damage and reduces risk of cancer. Calcium might be protective by effects on cell cycle.

Diet-genomic interactions in cancer seem likely to go beyond interactions with the normal genome and involve enhancement of normal cellular responses to DNA damage such that genome stability is more effectively maintained.

The interactive effect of methyl-group diets and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer

DH Kim¹ and YO Ahn², Department of Social and Preventive Medicine¹, Hallym University, Department of Preventive Medicine², Seoul National University

Some studies have shown stronger associations between combinations for high folate and low alcohol intakes, supporting a role of methyl group availability as an underlying mechanism for an effect of folate on colorectal carcinogenesis. A multicenter case-control study was conducted to see whether there is an interactive effect of 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphism and methyl group diets on the risk of colorectal cancer.

Cases were a consecutive series of patients with histologically confirmed, incident colorectal cancer who were admitted to two university hospitals and one general hospital in Seoul, Korea between 1998 and 2004, and controls were selected at the same hospitals as the cases. A total of 884 cases and 704 controls were enrolled. Subjects were genotyped for MTHFR by PCR-RFLP.

In the comparisons of the cross-classified combinations of total folate and alcohol consumption, the high risk groups (heavy drinker and lowest intake of folate) showed 2.52 (95% Confidence Interval [CI] 1.39-4.58) times greater risk of developing colorectal cancer compared to the low risk groups (non-drinker and highest intake of folate). Those with the TT genotypes of MTHFR had a reduced risk of colorectal cancer, compared with CC genotypes (adjusted odd ratio [aOR] 0.57, 95% CI 0.42-0.77). When the combined effects of MTHFR polymorphism and methyl group diets were assessed, the beneficial effect of TT genotypes was further strengthened among those with high methyl diets (aOR 0.30, 95% CI 0.14-0.64), while higher risk of TT was observed among those with low methyl diets (p for interaction 0.04). In summary, the beneficial effect of TT genotype of MTHFR was further strengthened among those with high methyl-group diets.

The interactive effect of alcohol drinking and polymorphism of acetaldehyde dehydrogenase on the risk of colorectal cancer

YO Ahn¹ and DH Kim², Department of Preventive Medicine¹, Seoul National University,
Department of Social and Preventive Medicine², Hallym University

Alcohol intake was reported to be a potentially modifiable behavior that may be related to colorectal cancer risk in the numerous epidemiologic studies. Acetaldehyde dehydrogenase (ALDH2) is a major enzyme responsible for the metabolism of acetaldehyde, oxidative product of alcohol, in the body. A multicenter case-control study was conducted to investigate the interactive effect of alcohol drinking and the ALDH2 polymorphism on the risk of colorectal cancer.

Cases were a consecutive series of patients with histologically confirmed, incident colorectal cancer who were admitted to two university hospitals and one general hospital in Seoul, Korea between 1998 and 2004, and controls were selected at the same hospitals as the cases. A total of 884 cases and 704 controls were enrolled. Subjects were genotyped for ALDH2 by PCR-CTPP.

After adjusting for potential covariates, the GA or AA genotype of ALDH2 was not associated with the overall risk of colorectal cancer (adjusted odd ratio [aOR] 1.02, 95% confidence interval [CI] 0.82-1.26 for GA genotype; aOR 0.64, 95% CI 0.33-1.24 for AA genotype) compared with the wild type. Compared with nondrinkers, aORs were 1.37 (95% CI 0.98-1.90) for those who consumed 30- <60 gram/day of ethanol and 1.66 (95% CI 1.24-2.22) for those who consumed \geq 60.0 gram/day of ethanol (p for trend 0.004). When stratified by genotypes of ALDH2, the effect of heavy drinking (\geq 60 gram/day) was stronger among those with variant A alleles of ALDH2 (OR 4.27, 95% CI 1.50-12.2) than among those with wild type (OR 1.39, 95% CI 0.87-2.21). The combined effect of ALDH2 polymorphisms and alcohol drinking was also statistically significant (p for interaction < 0.05). In summary, the adverse effect of heavy drinking on colorectal cancer risk was enhanced among those with inactive ALDH2 alleles.