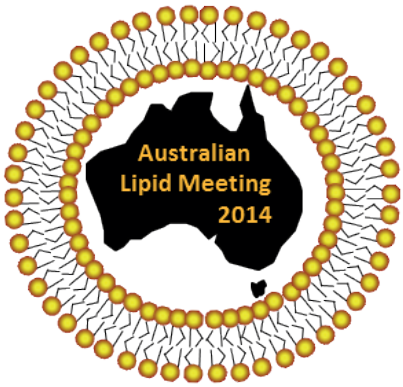

2ND AUSTRALIAN LIPID MEETING

IN CONJUNCTION WITH OMEGA-3 SYMPOSIUM

December 3-5, 2014
University of Wollongong - Innovation Campus
Wollongong, NSW, Australia

Sponsors

The organising committee wishes to acknowledge the following sponsors for their generous support.

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Contents

Welcome

On behalf of the organising committee welcome to Wollongong and the 2nd Australian Lipid meeting (ALM) co-hosted with the Omega-3 Centre. We are pleased to present an opportunity to discuss all aspects of lipid research and this year's meeting expands on the first Lipidomic's Users meeting held at Bio 21, Melbourne in January 2013. Lipid research continues to grow and strengthen and we hope to facilitate this with future Australian Lipid meetings.

We would like to take this opportunity to thank our sponsors whose generous support contributes to the success of our meeting. We look forward to 3 days of rigorous scientific exchange, together with the strengthening of friendships and collaborations both old and new. We look forward to meeting you all and hope you have chance to sample all that Wollongong and the Illawarra has to offer.

Organising Committee

- **A/Prof Todd Mitchell** – Chair (Illawarra Health and Medical Research Institute, University of Wollongong)
- **A/Prof Barbara Meyer** - (School of Medicine, University of Wollongong)
- **Prof Paul Else** - (School of Medicine, University of Wollongong)
- **Dr Simon Brown** - (Illawarra Health and Medical Research Institute, University of Wollongong)
- **Dr Andrew Jenner** - (Illawarra Health and Medical Research Institute, University of Wollongong)
- **Dr Sarah Abbott** - (Illawarra Health and Medical Research Institute, University of Wollongong)
- **Dr Thusitha Rupasinghe** - (Metabolomics Australia, University of Melbourne).

Conference Information

Venue

The conference will be held at the Australian Institute for Innovative Materials (AIIM) building (bld 236 - enter via bld 231) located at the Innovation Campus of the University of Wollongong Map 1 and 2). For general information the Innovation Campus office Phone is: + 61 2 4221 5115

Welcome Reception will be held in the Innovation Campus iC central Building (bld 230) at 5:30 pm on Wednesday 3rd December .

Oral sessions will all be presented in the lecture hall located in the Leone Kane-Maguire theatre on the first floor of the AIIM building.

Posters will be displayed throughout the meeting on the ground floor of the AIIM building, below the lecture hall.

Audio Visual for presenters

Please load your slides onto the venue computer during the break prior to the session you will be presenting in at the very latest. The lecture hall contains a modern suite of audio visual equipment, but presenters are advised to use PC or Mac. Please make early arrangements if you require special audio visual requirements

Poster sessions

Posters will be displayed throughout the meeting on the ground floor of the AIIM building (bld 236), below the lecture hall sessions. Numbered poster boards corresponding to each abstract and fixing material will be available at the start of the conference. Presenters are invited to attend their posters on Thurs lunchtime (12:20 – 1:20 pm). Delegates are asked to remove their posters by Fri lunchtime.

Name Badges

Delegates are requested to wear name badges at all times during the conference, as they are required for entry to sessions, morning and afternoon teas and lunches as well as the conference welcome reception and dinner.

Social Programme

Welcome Reception

The welcome reception including drinks and canapes, will be held 5:30 – 7:30 pm on Wednesday 3rd December, following the ALM Opening plenary lecture in the iC Central atrium

Conference Dinner

The conference dinner will be held from 7:00 – 11:00 pm on Thursday 4th December at the Harbourfront Restaurant, located at Belmore Basin Wollongong (see Map 1)

General Information

Eating out

There are plenty of options in the Wollongong area for eating out. For dinners we recommend Wollongong CBD for a variety of restaurants. Several are located along Keira St and Kembla St. (Map 1)

Coffees, teas and lunches are provided during the conference. Inside the Innovation Campus, The Matchbox and iC Kiosk are open from 7:15 and 8:00 am respectively, offering a variety of coffee, tea, fresh sandwiches, pastries, wraps etc.

Banking

There are no ATM facilities available at the Innovation Campus. The nearest ATMs are located at a variety of banks in Wollongong CBD or Fairy Meadow shopping centre.

Mobile phones and Internet

We kindly request that you have phones and other IT devices switched off or in silent mode during the conference sessions to minimise interruptions.

Delegates may use the 'eduroam' network (http://www.eduroam.edu.au) for internet access, or alternatively the visitor network with details below.

Login: UOW_EVENT3

Password: u0walm14

Pharmacy

The nearest pharmacies are located in Wollongong CBD or Fairy Meadow Shopping Centre.

Parking

Parking Fees for the Innovation Campus short-term parking station (Map 3) apply Monday to Friday: 8.30am - 6.30pm.

0-30 Minutes FREE, 30 Minutes - 1 Hour $2.00, 1 - 2 Hours $4.00, 2 - 4 Hours $6.50, 4 - 6 Hours $7.80, Over 6 Hours $13.00

Public Transport

The Innovation Campus is well-served by public transport, including a free shuttle bus service which connects with services on Cityrail's South Coast line. Transport Infoline: 131 500 or www.131500.com.au

Rail -The Innovation Campus is located between North Wollongong and Fairy Meadow railway stations. However, if you are travelling by rail to the Innovation Campus it is best to disembark at Wollongong railway station and catch the free Gong Shuttle service to the Campus.

Bus -The Free Gong Shuttle service (No 55) operates on a loop that connects the Innovation Campus with the University of Wollongong's main campus, Wollongong Hospital, Wollongong railway station, the city centre, North Wollongong beachside precinct, and the Fairy Meadow railway station. Buses travel in both directions on the loop. On weekdays the service operates every 10 minutes between 7am and 6pm and every 20 minutes between 6pm and 10pm. visit http://www.uow.edu.au/transport/shuttles/index.html for more information. The nearest bus stop to the conference venue just 100m away on Squires way

Private Transfers

Please contact the following private transfer companies in Wollongong to arrange transport:

By the Sea Luxury Cars – Ph: 0419 427 882 email: possie@tpg.com.au

Super Hire Cars – Ph: 02 4228 3635 email: enquiries@superiorhirecars.com.au

Wollongong Limousine Service – Ph: 02 4225 3666

Shuttle Bus: Leisure Coast Limousine Services and Airport Connections also offer transportation to/from Sydney Airport and Sydney City. Services details are available on Leisure Coast Limousine Services and Airport Connections website.

Cycleway

The Innovation Campus is also located on the main north-south cycleway that is part of Wollongong City Council's 42km cycleway network

Activities/sightseeing

There are many tourist activities and interesting sites to visit around Wollongong and the Illawarra region (http://visitwollongong.com.au). The Illawarra coast has dramatic views of the sea, with long ocean drives and pretty beaches along the way. Lookout points on the escarpment provide grand views of Wollongong and the coastal strip.

Plenary Speakers

Prof William Harris

Dr Harris is currently Professor of Medicine at the Sanford School of Medicine, University of South Dakota. He is also the founder and President of OmegaQuant Analytics, LLC (Sioux Falls) and a Senior Research Scientist at Health Diagnostic Lab. Since 1980, his research has focused primarily on omega-3 fatty acids and cardiovascular disease publishing over 150 scientific papers on the topic. His work has been central in establishing the importance of omega-3 fatty acids in cardiovascular health and with Prof Clemens Von Schacky he invented and defined the HS-Omega-3 Index®.

Mr Ian Newton

Mr Newton is the principal of Ceres Consulting and an internationally recognized expert in human nutrition. Mr Newton held various director positions in Hoffmann-La Roche and Roche Vitamins before establishing Ceres Consulting in 2004. He is a member of the Council for Responsible Nutrition, the American Oil Chemists Society, and the Institute of Food Technologists. He is on the Advisory Committee of The Richardson Centre for Functional Foods & Nutraceuticals at the University of Manitoba and serves on the editorial advisory boards of Nutrition Business Journal and Functional Foods & Nutraceuticals Journal

A/Prof Markus Wenk

Markus Wenk is currently Associate Professor of Biochemistry at the National University of Singapore (NUS), director and founder of the Singapore lipidomics incubator (SLING) and Privatdozent at the University of Basel. He is also an executive editor of Progress in Lipid Research. His research utilizes novel approaches in systems scale analysis of lipids and their interactors (lipidomics) and is he recognized as one of the thought leading investigators worldwide in this emerging field.

A/Prof Ute Roessner

A/Prof Roessner is an Australian Research Council Future Fellow and head of the Australian Centre for Plant Functional Genomics (www.acpfg.com.au) and the Metabolomics Australia (www.metabolomics.com.au) nodes at the School of Botany, The University of Melbourne, Australia. She is a leader in in the field of plant metabolomics and has developed novel GC-MS and LC-MS techniques for the analysis of plant metabolites. The main focus of A/Prof Roessner's current research is to identify novel mechanisms of salinity tolerance in barley by spatial analysis of metabolites and lipids using Imaging Mass Spectrometry.

Prof Katharina Gaus

Prof Katharina Gaus is head of the Cellular Membrane Biology Laboratory at the University of New South Wales. The main aim of her research has been to gain a mechanistic understanding of the organisation of the plasma membrane within cells. She has pioneered fluorescence microscopy approaches to examine and quantify cell signalling on a single molecule level (super-resolution microscopy) in living cells. Her work aims to link membrane organisation to cell signalling by implementing single-molecule imaging techniques and using novel cell-activating surfaces.

Prof Trevor Mori

Prof Mori is a Winthrop Professor and Senior Research Fellow of the National Health and Medical Research Council of Australia in the School of Medicine and Pharmacology, at the University of Western Australia. His research is primarily associated with studying the effects of diet, particularly dietary omega-3 fatty acids, and lifestyle, on risk factors for cardiovascular disease (CVD); the role of lipid oxidation in atherosclerosis, CVD and clinical medicine; and cardiometabolic risk factors in the Western Australian Pregnancy (Raine) Study, a pregnancy cohort study of 2,800 participants. Other research interests include fatty acid metabolism; anti-inflammatory omega-3 fatty acid-derived resolvins and protectins; platelet and leukocyte function; and control mechanisms in blood pressure regulation.

A/Prof Christer Ejsing

A/Prof Ejsing is the head of the lipidomics laboratory at the University of Southern Denmark. His research focuses on the development and application of mass spectrometry-based lipidomics techniques that allow global analysis of cellular lipidomes with the ultimate goal of further understanding cell physiology. This work includes designing new comprehensive lipidomics strategies for delineating the regulatory circuit of lipid metabolism in simpler systems (e.g. *S. cerevisiae*). These techniques and tools are extended to workflows for multiple-cellular organisms paving the way for a systems wide approach to understand the regulation of metabolism in health and disease.

Prof Andrew Brown

Prof Brown is currently head of the School of Biotechnology and Biomolecular Sciences at the University of New South Wales. His research focusses on the cellular regulation of cholesterol levels in both physiological and pathophysiological states such as cancer. His work has shed new light on cholesterol synthesis and metabolism and has uncovered an important link in the cell signalling processes of cholesterol metabolism and cancer.

Omega-3 Symposium and ALM Program

Wednesday December 3

9:00am	Omega-3 & ALM2 Registration (open all day)
	Omega-3 Symposium welcome:
10:00 am	The "Good Science" behind long-chain omega-3
	Omega-3 Session 1 Chair: Craig Patch
10:05 am **O1**	Omega-3 index: How biomarkers help clarify the omega-3 story. **William Harris** (U of South Dakota, USA)
10:50 am	Morning Tea
	Omega-3 Session 2 Chair: Barbara Meyer
11:20 am **O2**	Fatty acid desaturase 2 (FADS2) and 1 (FADS1). Novel functions in highly unsaturated fatty acid (HUFA) biosynthesis. **Thomas Brenna** (Cornell, USA)
11:50 am **O3**	Variability - the great offender in omega-3 science. Methodological considerations towards improving meaningfulness of human studies. **Giovanni Turchini** (Deakin, Australia)
12:20 pm	Lunch
	Omega-3 Session 3 Chair: Barbara Meyer
1:20 pm **O4**	Global Update on Omega-3 Ingredients and Science. **Ian Newton** (Ceres Consulting, Canada)
2:05 pm **O5**	Metabolic Engineering of Plant Oils: Yield and Omega-3. **James Petrie** (CSIRO, Australia)
2:35 pm	Afternoon Tea
	Omega-3 Session 4 Chair: Craig Patch
3:00 pm **O6**	Developments, issues and future directions with long-chain Omega-3: an Australian perspective. **Peter Nichols** (CSIRO, Australia)
3:30 pm **O7**	The role of omega-3 fatty acids in pregnancy and early life. **Robert Gibson** (U of Adelaide, Australia)
4:00 pm	Omega-3 Close
4:30 pm	**ALM2 Welcome**
	ALM2 Opening Plenary Chair: Todd Mitchell
4:40 pm **A1**	Natural variation of lipidomes. **A/Prof Markus Wenk** (NUS, Singapore)
5:30 pm	Welcome Reception: iC Central Atrium

ALM Program Day 2

Thursday December 4

	Session 1: Plants Chair: Thusitha Rupasinghe
9:00 am **A2**	Identifying novel salinity tolerance mechanisms by spatial analysis of lipids in barley roots **Ute Roessner** (U of Melbourne, Australia)
9:30 am **A3**	A new plant oil production platform: seed-like oil yield from biomass. **James Petrie** (CSIRO, Australia)
9:50 am **A4**	Super high oleic safflower: development and lipidomic analysis of an engineered near-pure source of industrial oil. **Mathew Taylor** (CSIRO, Australia)
10:10 am **A5**	The flagellar membrane of chlamydomonas is a specialized, highly ordered Lipid domain of the plasma membrane enriched in raft lipids. **Antonio Castillo-Flores** (U Mass, USA)
10:30 am	Morning Tea
	Session 2: Imaging/labelling Chair: Damien Callahan
11:00 am **A6**	Single molecule microscopy for membrane biology and T cell signalling. **Katharina Gaus** (UNSW, Australia)
11:30 am **A7**	Lipidomic profiling of malignant brain tumour in mouse brain parenchyma. **Hay-Yan J Wang** (NSYSU, Taiwan)
11:50 am **A8**	Tracer lipidomic analysis reveals alterations in rodent hepatocyte phosphatidylcholine and phosphatidylethanolamine metabolisms after fatty acid treatment. **Kang-Yu Peng** (Baker IDI, Australia)
12:10 pm	Lunch & Poster Session

	Session 3: Nutrition Chair: Jennifer Saville
1:40 pm **A9**	Omega-3 fatty acids and cardiovascular disease: Effects on cardiometabolic risk factors. **Trevor Mori** (U Western Australia, Australia)
2:10 pm **A10**	Unique changes in hepatic sphingolipid species after high-fat feeding in Balb/c mice correlate with protection from diet-induced glucose intolerance. **Magdalene Montgomery** (UNSW, Australia)
2:30 pm **A11**	F4 –neuroprostanes, non enzymatic metabolites of DHA are responsible of the anti-arrhytmic properties of DHA. **Thierry Durand** (U Montpellier, France)
2:50 pm **A12**	Investigation of stability of protein-oleic acid complexes having anti-cancer activity. **Emma Rath** (U Sydney, Australia)
3:10 pm	Afternoon Tea
	Session 4: Technical Developments & Methodology Chair: Simon Brown
3:40 pm **A13**	Functional lipidomics: from structural characterization to regulation of lipid metabolic networks. **Christer Ejsing** (U Southern Denmark, Denmark)
4:10 pm **A14**	Developing next generation technologies for unmasking the lipidome: New tools to address the challenge of lipid isomers. **Stephen Blanksby** (QUT, Australia)
4:30 pm **A15**	Profiling of phospholipid classes using 31p NMR. **Andrew Mackenzie** (Callaghan Innovation, New Zealand)
4:50 pm **A16**	Isobaric mass tagging and targeted MS/MS for multiplexed quantification and characterization of aminophospholipids in complex lipid mixtures. **Gavin Reid** (U Melbourne, Australia)
5:10 pm **A17**	Resolution of lipid isobars by mass spectrometry using differential mobility separation (DMS). **Paul Baker** (AB Sciex, USA)
7:00 pm	Conference Dinner: Harbourfront Restaurant

ALM Program day 3

Friday December 5

	Session 5: Health and Disease Chair: Christopher Barlow
9:00 am **A18**	The ups and downs of cholesterol homeostasis. **Andrew Brown** (UNSW, Australia)
9:30 am **A19**	A lipidomic study of ceramide metabolism in the liver and its role in two lysosomal lipidoses: drug-induced phospholipidosis and sandhoff disease. **John Shockor** (Waters, USA)
9:50 am **A20**	Protein kinase C epsilon deletion in adipose tissue, but not in liver, modulates fatty acid mobilisation and hepatic insulin action. **Carsten Schmitz-Peiffer** (Garvan, Australia)
10:10 am **A21**	Plasma lipid profiling to predict cardiovascular events in type 2 diabetes. **Peter Meikle** (Baker IDI, Australia)
10:30 am	Morning Tea
	Session 6: Hot topics Chair: David Harman
11:00 am **A22**	A more robust lipidomics workflow for high-resolution LC-MS and data dependent MS-MS using a high-field Orbitrap mS and Lipid-Search software. **David Peake** (Thermo Scientific, USA)
11:20 am **A23**	Lipid synthesis and storage in human metabolic disorders. **Hongyuan Robert Yang** (UNSW, Australia)
11:40 am **A24**	Towards determination of natural variation of blood lipids in healthy individuals. **Husna Begum** (Baker IDI, Australia)
12:00 pm **A25**	Loss of neuroprotective sphingosine 1-phosphate in the early stages of Alzheimer's disease. **Nupur Kain** (UNSW, Australia)
12:20 pm **A26**	Of mice, pigs and men: shotgun-lipidomics analysis of mitochondrial phospholipids from mammals with very different lifespans. **Colin Cortie** (U of Wollongong, Australia)
12:40 pm	**ALM2 close**

Abstracts of Oral Presentations

O1 OMEGA 3 INDEX: HOW BIOMARKERS HELP CLARIFY THE OMEGA-3 STORY

William S. Harris

[1]*Department of Internal Medicine, University of South Dakota School of Medicine and OmegaQuant Analytics, LLC, Sioux Falls, SD; Health Diagnostic Laboratory, Richmond, VA, USA*

The Omega-3 Index is the sum of EPA+DHA in RBC membranes expressed as a percent of total fatty acids. Typical levels range from 3% to 9%, with a US average value of between 5%-6%. The Index is a marker of tissue EPA+DHA and therefore reflects an individual´s EPA+DHA status. The Omega-3 Index currently fulfills most of the criteria for a cardiovascular risk factor. In routine clinical practice, the Omega-3 Index can be used to assess baseline n-3 fatty acid status and to check for compliance with a recommendation to increase the n-3 fatty acid intake. In the research setting, the Omega-3 Index can likewise document compliance, both to confirm that those assigned to the n-3 fatty acid group followed instructions and that those assigned to placebo did not take n-3 fatty acid off protocol. In addition, the Omega-3 Index can be used as an inclusion criterion in trial design to confirm that only individuals most likely to benefit from n-3 fatty acid treatment are included. A biomarker like the Omega-3 Index is superior to diet-record-based estimates of omega-3 intake for prospective cohort studies and can uncover disease relationships with omega-3 status that may not be discerned using food frequency questionnaires. In summary, the use of the Omega-3 Index in research will help clarify the role(s) that these fatty acids play in human health, and its use in the clinic will allow clinicians to detect omega-3 “insufficiency”, to stratify patients with respect to risk for disease, and to titrate their omega-3 levels into a protective zone. Wider implementation of the Omega-3 Index could ultimately reduce the burden of cardiovascular and possible neuropsychiatric diseases.

O2 FATTY ACID DESATURASE 2 (*FADS2*) AND 1 (*FADS1*). NOVEL FUNCTIONS IN HIGHLY UNSATURATED FATTY ACID (HUFA) BIOSYNTHESIS

J.Thomas Brenna and Kumar S.D. Kothapalli
Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA

Conversion of C18 polyunsaturated fatty acids (PUFA) into HUFA is normally depicted as a competitive, linear biochemical pathway, in which 6-desaturation is often referred to as rate limiting and existing only in the endoplasmic reticulum (ER). Molecular, isotope tracer, and specific structural mass spectrometry techniques reveal that the gene product of fatty acid desaturase 2 (*FADS2*, 11q12.2) has at least 7, and probably 9, fatty acid substrates. We review evidence for all these substrates and present original evidence that *FADS2* 4-desaturates 22:5n-3 and 22:4n-6. For *FADS2* studies, yeast and a human breast cancer cell line lacking 6-desaturase activity, MCF7 cells, are our primary *in vitro* models. *FADS2*, but not *FADS1,* gains activity toward all these substrates, while *FADS1* transfection causes gain of 5-desaturase activity toward 20:3n-6 and 20:4n-3. Yeast transformed with *FADS2* gain activity toward 20:2n-6 and 20:3n-3 to yield 20:3n-6 (DGLA) and 20:4n-3, both eicosanoid precursors. Stably transfected MCF7(*FADS1*) cells incubated with 20:2n-6 and 20:3n-3 synthesize 5,11,14-20:3 and 5,11,14,17-20:4, respectively, presumably by action of native and transfected *FADS1*, similar to *FADS2*-null mice and wild type *FADS2*-null cats *in vivo*. MCF7(*FADS2*) cells have normal 6 and 8 desaturase activity. All MCF7 cells incubated with 22:6n-3 or 22:5n-6 accumulate, respectively, 24:6n-3 and 24:5n-6 in a dose response manner indicating that both substrates are precursors of the respective C24 PUFA. MCF7(*FADS2*) cells incubated with 22:4n-6 accumulate 22:5n-6 before any 24:5n-6 is detected, and MCF-7(*FADS2*) cells incubated with 20:5n-3 (EPA) or 22:5n-3 accumulate 22:6n-3 (DHA). When incubated with d5-18:3n-3, MCF7(*FADS2*) accumulate d5-22:6n-3 before any label can be detected in 24:5n-3 or 24:6n-3 similar to data of others establishing 4-desaturation as the last step in DHA synthesis in two vertebrate fish and in dinoflagellates. Strong attenuation of rodent tissue DHA accumulation by high 18:2n-6 feeding ascribed to double use of *FADS2* is consistent with competition of 18:2n-6 for *FADS2* mediated 4-desaturation, as well as the presumed 6-desaturation of 24:5n-3. *FADS2* mediates desaturation at three (4 6,8) positions, specific to each of 8 different PUFA and one saturated fatty acid.

O3 VARIABILITY - THE GREAT OFFENDER IN OMEGA-3 SCIENCE. METHODOLOGICAL CONSIDERATIONS TOWARDS IMPROVING MEANINGFULNESS OF HUMAN STUDIES.

Giovanni M. Turchini [1], Samaneh Ghasemifard [2], and Andrew J. Sinclair [2]
[1] *School of Life and Environmental Sciences, Deakin University, Warrnambool, VIC, Australia;* [2] *Metabolic Research Unit, School of Medicine, Deakin University, Geelong, VIC, Australia*

The fatty acid composition of dietary doses and target tissues are commonly the principal outcome of studies focusing on omega-3 fatty acids. Nevertheless, reviewing available publications on omega-3 in human intervention trials, it appears that methodological considerations relative to how this outcome is achieved are often overlooked. More often than not, studies report lack of statistical significance, even for values that from a physiological point of view could likely be extremely significant. These observations can simply be summarised as typical examples of type II error, fundamentally driven by low power of the statistical test. For any test, the statistical power depends primarily on the sample size and the variability of the target variable. Animal studies, which compared to human studies have the logistic advantage of more easily increase sample size and reduce variability by carefully controlling possible indirect sources of statistical dispersion, are currently responsible for generating robust outcomes and remarkable scientific advancements, whilst human studies are struggling to keep the same pace.

This paper is not about statistic, but on the basis of these simple statistical concepts and after reviewing the methodological approaches commonly used in omega-3 studies in humans, we present a series of the most commonly overlooked methodological considerations. These methodological considerations span from the study design, such as doses administered and body weight, age and gender of participants, to analytical procedures, such as target tissues, laboratory protocols, chromatography conditions, incomplete fatty acid reporting and transformation, and the erroneous use of fatty acid percentages. All these methodological constraints contribute directly to the large inter-individual variability observed in studies' outcomes.

Whilst it is evident that increasing sample size is often extremely challenging in human studies, the reduction of variability appears to be more easily implemented and thus, an effective strategy towards improving the meaningfulness of omega- 3 studies in humans. This paper will laid out a set of criteria and suggestions for conducting future studies on omega-3 in humans.

O4 A LOOK AT THE OMEGA-3 MARKET AND IMPLICATIONS FOR THE FUTURE.

Ian Newton,
Ceres Consulting, 29 Taurasi Court, Suite 201, Markham, Ontario, Canada. L6E 1T7

An overview of the omega-3 market, how it is solidly based on forty years of science, which even today continues to expand and add many new future opportunities.

The market, despite showing some slowing in the last twelve months or so, continues globally in 2014 at a double digit rate. All sectors including functional foods, pharmaceutical use, and infant formulae show growth. Globally China and the EU lead the way particularly in the functional food sector; however dietary supplement use still dominates both in revenues and tonnage.

Concern over the sustainability of anchovy stocks continues, but alternate sources are entering the market and crop sources of omega-3 oils are now in view.

The main drivers of growth future growth, apart from the ever growing publication of scientific papers are the need for Reference Daily Intakes (RDA's, RDI's) and government recommendations. Notably absent is the lack of clear RDI's in the USA and Canada, but an Institute of Medicine review on lipids room and may be expected in 2015/6, which could eventually lead to official recommendations.

In addition to RDI's, and despite overwhelming data on the health benefits for omega-3's, is the need for government sanctioned health claims and labelling that will educate consumers on the benefits, for CVD, vision, brain and joint and neurological health.

Finally governments ever burdened with healthcare expenditures are looking for proven actives to help their aging populations maintain health and reduce costs. Several analyses point to omega-3's with their well known benefits as being useful in reducing costs in aging western societies.

O5 **METABOLIC ENGINEERING OF PLANT OILS: YIELD AND OMEGA-3**

James R. Petrie [1], Thomas Vanhercke [1], Surinder P. Singh [1]
[1] *CSIRO Food, Nutrition and Bioproducts Flagship, ACT, Australia*

Demand for plant oils will increase rapidly as the population grows in the coming decades. Limitations on arable land and other inputs mean it may be difficult to meet this additional demand with current oilseed-based production systems. There is also significant growing demand for specific fatty acids such as the long-chain omega-3 EPA and DHA. This talk will describe recent plant engineering efforts at CSIRO aimed at both plant oil yield (biomass oil) and fatty acid quality (DHA canola). First, we have produced plants in which over 30% of the leaf (dry mass) is triacylglycerol. This was achieved by combining multiple oil increase technologies in a coordinated approach to overcome the 'Push' (fatty acid synthesis), 'Pull' (TAG assembly) and 'Protect' (oil storage) limitations in plant cells. When considered in the context of a high biomass crop species this has the potential to provide a new oil production pathway.

This talk will also describe the engineering of long-chain omega-3 fatty acid in plant seeds. These fatty acids are currently predominantly sourced from fish and algal oils. However, in order to meet the increasing demand for these oils there is an urgent need for an alternative and sustainable source of EPA and DHA. We have focused on maximising the production of DHA in seed. This talk will also describe the transition of DHA production in seed of our model species Arabidopsis through to *Camelina sativa*, *Brassica juncea* and our target crop *Brassica napus*. DHA levels that exceed the amount typically found in bulk fish oil have now been achieved in all four species. This talk will describe constructs, transgenic plants and seed oil fatty acid profiles. We will also describe the progress of GM canola trials that are currently in the field.

O6 DEVELOPMENTS, ISSUES AND FUTURE DIRECTIONS WITH LONG-CHAIN OMEGA-3: AN AUSTRALIAN PERSPECTIVE

Peter D Nichols
CSIRO Food, Nutrition & Bioproducts; Oceans & Atmosphere Flagships, Hobart, Tas 7000, Australia

Substantial evidence exists on the health benefits of the long-chain (LC ≥c20) omega-3 oils, in particular EPA (eicosapentaenoic acid, 20:5w3) and DHA (docosahexaenoic acid, 22:6w3). Notwithstanding, current issues in this still growing research area and also its markets include: the diminishing fish oil resource against the expanding market demand, competition for the resource (e.g. aquafeed versus higher value supplements and pharmaceucticals), the decreasing product quality in farmed seafood in terms of their LC omega-3 content, the different role of short-chain (≤c18) and LC omega-3 and resulting consumer confusion, the need for new and sustainable sources of LC omega-3, the recent and on-going negative media towards LC omega-3, analytical issues with specific emphasis on accurate measurement of LC omega-3, the future role for DPA (docosapentaenoic acid, 22:5w3), and various debates on both the form of (triacylgycerol, ester, polar lipid) and source (fish, krill, squid). These topics will be discussed with an emphasis on Australian case studies. The much needed advisory needs including for progressing developments in many of these areas sees considerable inputs in North America and Europe from the international body GOED (global organization for EPA and DHA). In Australia and New Zealand the omega-3 center including its scientific advisors and committee play a lead advisory role with LC omega-3 oils.

O7 THE ROLE OF OMEGA 3 FATTY ACIDS IN PREGNANCY AND EARLY LIFE

Robert A Gibson[1]

[1]*NHMRC Research Fellow, Director FOODplus Research Centre, University of Adelaide, Adelaide, Australia*

The metabolic demand for omega-3 long chain polyunsaturated fatty acids, particularly docosahexaenoic acid (22:6 omega-3, DHA), is increased during pregnancy because of the extra needs of the fetus, expanded maternal cell mass and placenta. However, maternal dietary DHA intake in pregnancy is low and it is not clear whether adaptive metabolic mechanisms are capable of meeting the increased DHA need in pregnancy. What is clear is that preterm infants are at greater risk of DHA depletion than their term born counterparts because they are born before they have had the opportunity to accumulate a full complement of DHA. This is well illustrated through our recent trials, one involving preterm infants (the DINO trial, n=650) and another involving pregnant women who largely gave birth to term infants (the DOMInO trial, n=2400). The DINO trial showed that supplementation which aimed to achieve the concentration of DHA accumulated in the womb resulted in fewer infants with mild and significant cognitive delays at 18 months of age, although there were no differences in the mean developmental quotient scores.

In the DOMInO trial we observed one of the most consistent clinical effects of omega 3 fatty acids, an extension in the length of gestation. These results were confirmed in the recent KUDOS trial from the United States. Both DOMInO and KUDOS demonstrated a marked reduction in the incidence of early preterm birth. The usefulness of DHA to prevent preterm birth in a large population (n=5500) is currently being evaluated.

The most important measure of the well-being of preverbal children is growth. Both of our large studies, DINO and DOMInO have shown that supplementation of the diet with relatively high levels of DHA has almost no effect on growth, despite the fact that the level of DHA exceeded the level of AA in the diet. Further work is needed to better define the sub-groups of children who will benefit from DHA supplementation during the perinatal period.

A1

NATURAL VARIATION OF LIPIDOMES

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Once viewed simply as a reservoir for carbon storage, lipids are no longer cast as bystanders in the drama of biological systems. The emerging field of lipidomics is driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells (Wenk 2010 Cell 143(6):888-95).

Our recent results show extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans (Chua et al 2013 Proc Natl Acad Sci U.S.A. 110(35):14468-73). I will also introduce a strategy for capture of phospho-monoester lipids. Using this enhanced workflow we identified novel forms of sphingosine-1-phosphates, in tissue from human, mouse and fruit fly, respectively.

Understanding better the fundamentals of natural variation in lipidomes as well as specific recognition of individual lipid species are the scientific aims of SLING, the Singapore Lipidomics Incubator (http://lsi.nus.edu.sg/corp/research-programmes/sling/). This centre is a global magnet for collaborating parties in lipidomics – from academia and industry – delivering new technologies and intellectual capital. SLING organizes the international Singapore Lipid Symposium (ISLS), a major symposium in lipidomic research in Asia Pacific (http://www.lipidprofiles.com/index.php?id=82) and 'i c lipid', an intensive immersion course in mass spectrometry based lipidomics.
(http://www.lipidprofiles.com/index.php?id=139).

A2 IDENTIFYING NOVEL SALINITY TOLERANCE MECHANISMS BY SPATIAL ANALYSIS OF LIPIDS IN BARLEY ROOTS

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We are facing the challenging task to meet the growing demand for food which must occur in an environment of a changing climate with increasing environmental stresses such as drought, extreme temperatures, nutrient deficiencies and mineral toxicities. Less land available to cultivate crops, declining water quality and prioritization of biofuel production at the expense of food production further exacerbates the situation. A combination of climate change and poor agricultural practices signifies that 50% of current arable land is at high risk of increased salinity and hence unusable by 2050. Here we aim to develop and apply new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signaling is an integral part of the complex regulatory networks in the responses of plants to salinity. Modifications of membrane lipids occur through the activity of phospholipases, lipid kinases and phosphatases such as phospholipase D and diacylglycerol kinase that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and differentiation and for an appropriate response of the plant to environmental stimuli. We are using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. Our aim is to investigate the link between PM composition and functionality in aspects of salinity response by examining whether observed changes in lipids are involved in either the alteration of fluidity, or in lipid-based downstream signaling. We are also using MALDI-FT-MS based imaging technologies to monitor spatial distributions of lipids across root sections of salt treated tolerant and intolerant barley genotypes. These novel findings will lead to a better understanding of the role of lipids, lipid composition and signaling for plant salt tolerance.

A3 A NEW PLANT OIL PRODUCTION PLATFORM: SEED-LIKE OIL YIELD FROM BIOMASS

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Supply of vegetable oils as a major commodity faces continuous pressure. Global demand is expected to double in the next two decades due to increasing world population and rising petroleum prices. Increasing limitations on arable land and agricultural inputs mean it will be difficult to meet this additional demand with current oilseed-based production systems. The concept of producing oil in the leaves and stems of high biomass species has attracted attention as a way to intensify oil production. The engineering of such a new oil production platform would not only yield greater amount of oil for a given land area but also provide a way to more easily segregate bioeconomy traits such as unusual fatty acids away from food production. We previously reported the accumulation of up to 17% triacylglycerol (TAG, dry weight) in leaf tissue of Nicotiana species. This was achieved by combinatorial metabolic engineering in which we increased fatty acid biosynthesis ('Push') by limited overexpression of the WRI1 transcription factor, increased TAG assembly ('Pull') by expressing DGAT1, and encouraged oil body formation ('Packaging') by expressing oleosin in plant leaves.

In this presentation, we will describe some of our second generation construct designs which have more than doubled the previously reported TAG content. Oil content in leaves now matches elite oilseed crop seed levels. We will describe the implications that this technology has for global plant oil production from a yield and intensification perspective, as well as the challenges that remain for integration into the existing industry. We will also present data demonstrating that the newly produced fatty acids can be modified for industrial or nutritional applications as well as preliminary data of a transcriptome comparison between wild type and high oil leaf tissue, harvested at different stages during plant development.

A4 SUPER HIGH OLEIC SAFFLOWER: DEVELOPMENT AND LIPIDOMIC ANALYSIS OF AN ENGINEERED NEAR-PURE SOURCE OF INDUSTRIAL OIL

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Plant oils potentially provide a scalable, sustainable and cost-competitive source of industrial feedstocks that are derived currently from non-renewable petroleum sources which are environmentally-damaging and increasingly expensive. However, plant oils currently suffer the disadvantage of predominantly being composed of a mixture of fatty acid structures, rather than a source of high purity compounds that are required for cost-effective oleochemical processing. At high purity oleic acid ($C18:1^{\Delta 9}$) is resistant to oxidative damage and can also be readily converted to a range of derivatives via reactive cleavage of the mid-chain double bond or olefin metathesis. Current safflower crops produce seed containing ~75% oleic acid, that is not suitable for oleochemical feedstock uses. We have used genetic engineering and RNAi silencing to specifically silence lipid desaturation pathways, FAD2, in safflower seed resulting in oils with ~93% oleic acid. These superhigh oleic acid oils are possibly suitable as feedstocks in lubricant and oleochemical formulations.

Here we report the detailed lipidomic analyses undertaken to fully characterise the lipid and oil composition of various safflower genotypes using tissues sampled from roots, leaves and seeds. These techniques included the use of triple quadrupole mass spectrometer to measure approximately 500 neutral loss experiments, enabling the determination of different acyl chain combination on numerous lipid species, spanning acyl-chain biogenesis and deposition into oils. While the distributions of oleoyl-related acyl chains in lipid pools differ significantly between the seeds, there are no significant differences in the roots and leaves of these different genotypes. This analysis provides evidence that the GM approach taken in the production of highly-pure oleic acid in safflower seed does not modify the oil composition in other non-target organs. Interestingly, a mutant of safflower containing a constitutive mutation in FAD2 was found to have significantly elevated levels of oleic acid in all tissues sampled, including ~89% in seed oil. In the roots of this mutant there was an accumulation of oleoyl-containing TAG species and an upregulation of elongated acyl chains indicating the presence of a functional fatty acid elongase. These results support the strategy of creating seed-specific modifications for the production of high purity oils whilst avoiding drastic changes in the metabolism in non-target organs.

A5 THE FLAGELLAR MEMBRANE OF CHLAMYDOMONAS IS A SPECIALIZED, HIGHLY ORDERED LIPID DOMAIN OF THE PLASMA MEMBRANE ENRICHED IN RAFT LIPIDS

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Cilia are essential organelles for human health. Although numerous studies have demonstrated that ciliary and flagellar membranes contain unique proteins and thus represent unique domains of the plasma membrane, no studies exist comparing the lipids of ciliary *vs*. plasma membranes. Because specific lipids may be important in sorting and targeting ciliary proteins at the trans-Golgi network, in intraflagellar transport, and in the functioning of membrane proteins, we are studying the lipidome of the *Chlamydomonas* flagellum. Wild-type and cell-wall mutant cells were grown under controlled conditions and deflagellated by treatment with dibucaine.

The lipid composition of whole cells, cell bodies, flagella, and cell body plasma membrane (PM) were isolated by two-phase partitioning and compared by mass spectrometry. The analyses showed that all compartments have distinct lipid profiles. The most abundant fatty acids (FAs) in flagella, representing 95% of the total, were the short-chain saturated palmitic (37%) and stearic (22%) acids, and the unsaturated oleic (15%) and γ-linolenic (21%) acids. γ-linolenic acid is highly enriched in flagella *vs*. to the PM (2%) and to cell bodies (5%). The ratio of saturated to unsaturated FAs in flagella was higher (1.5) than in cell bodies (0.4), but lower than in PM (7.4). The raft lipids phosphatidylethanolamine, ergosterol, stigmasterol, and β-sitosterol, and five unidentified lipids are enriched in flagella ~25-35x *vs*. to the PM, suggesting that the flagellar membrane is highly ordered, a prediction confirmed by *in vivo* membrane fluidity measurements by two-photon microscopy using the membrane polarity sensitive probe C-Laurdan. These results show for the first time that the flagellar membrane differs from the plasma membrane in lipid composition. The lipid raft-like composition of the flagellar membrane may have an essential role in the assembly and function of flagella.

A6 SINGLE MOLECULE MICROSCOPY FOR MEMBRANE BIOLOGY AND T CELL SIGNALLING

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While it is recognised that lipids and proteins must cooperate for receptor signalling, mapping the organisation of cellular membranes has been exceedingly challenging.

We have established single molecule localization microscopy to analyse the distribution of T cell signalling proteins in intact and live cells on the molecular scale. This has provided new insights into the mechanisms of Lck clustering (Rossy et al. Nat Immunol 2013) and LAT recruitment (Williamson et al. Nat Immunol 2011) upon activation of the T cell antigen receptor (TCR). However, super-resolution microscopy approaches for lipids are limited.

We are currently exploring the use of single molecule imaging to map membrane topography (Owen et al. Biophys J 2013) and fluorescence lifetime and spectral un-mixing to gain insights into the phase behaviour of lipids in cells (Owen et al. Nat Commun 2012).

A7 LIPIDOMIC PROFILING OF MALIGNANT BRAIN TUMOUR IN MOUSE BRAIN PARENCHYMA

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Brain tumours are among the leading cause of death of young children and adults. The highly invasive nature and the high recurrence rate of the brain tumour cells aggravate the difficulty in surgical resection and the options for the combined adjuvant therapy treatment. Identification of potential tumour marker panels not only aids in the differentiation of normal brain parenchyma from the tumour mass, it could also reveal underlying physiological differences and the potential therapeutic targets. Among all the potential markers, lipids appear to bear the nature that could effectively reflect the differences between these two tissue natures. To further investigate the potential of phospholipids as the biomarker of brain tumour, the GL261 mouse glioma cells were implanted in the brain parenchyma of B6 mice for lipidomic studies. After magnetic resonance imaging that confirmed the growth of implanted tumour cells, the mice brains were harvested and cut into coronal sections for MALDI-MS imaging of phospholipid distribution. In addition, the tumour region and the contralateral normal parenchyma were punctured for lipid extraction followed by qualitative and quantitative liquid-chromatography tandem mass spectrometry (LC-MS/MS) analyses of phospholipid content. MALDI-MS imaging of desalted brain sections revealed the tumour-specific phospatidylcholines, lysophosphatidylcholines, sphingomyelin that characterized the tumours in situ. The imaging results also revealed the lipids revealing the apoptotic nature at the tumour core region. The LC-MS/MS analysis confirmed the abnormal fatty acyl constituents on the tumour-specific phospholipids, and revealed the quantitative difference of various phospholipids between the malignant and the normal parenchyma. We expect that a lipid marker panel composed of these tumour-specific lipids and those showing abnormal expression in tumour could effectively distinguish the glioma mass from the normal parenchyma. Further investigation will evaluate the applicability of such lipid panel as the basis for the pathological grading of glioma tumours.

A8 **TRACER LIPIDOMIC ANALYSIS REVEALS ALTERATIONS IN RODENT HEPATOCYTE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE METABOLISMS AFTER FATTY ACID TREATMENT**

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Excessive accumulation of fatty acids in hepatocytes is a known factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). NAFLD is also associated with a change in the ratio of two major phospholipids, phosphatidylcholine (PC) to phosphatidylethanolamine (PE), in hepatocytes. PC synthesis primarily occurs via the addition of CDP-choline to diacylglycerol. Additionally, in hepatocytes significant conversion of PE to PC can occur via the sequential methylation of the phosphoethanolamine headgroup. This reaction is catalysed by phosphatidylethanolamine *N*-methyltransferase (PEMT), using *N*-adenosyl methionine as a methyl donor.

Here we have measured relative fluxes through the CDP-choline and PEMT pathways in primary and cultured hepatocyte lines with D_9-choline and D_3-methionine. We have also monitored PE synthesis via the CDP-ethanolamine pathway with D_4-ethanolamine labelling. All the labelled precursors were incorporated into PC in both the hepatoma cell line HepG2 and rodent hepatocytes after 8 hours. Additionally, we observed deuterium labelled lyso-PCs, lyso-PEs and sphingomyelins. In HepG2 cells the majority of PC synthesis occurred via the CDP-choline pathway with minimal contribution via the PEMT pathway. In contrast, in rodent hepatocytes we find that up to 14% of the newly synthesised PC arises from the PEMT pathway.

Treatment of primary hepatocytes with fatty acid (palmitate:oleate=1:2) results in a decrease in the PC/PE ratio. Flux analysis demonstrates an increase in PE synthesis through the CDP-ethanolamine pathway and a decrease in PC synthesis via the PEMT pathway. These results suggest that excessive fatty acid alters the flux through these two pathways, and this maybe a key driver in the reduction of the PC/PE ratio associated with the NAFLD type phenotype.

A9

OMEGA-3 FATTY ACIDS AND CARDIOVASCULAR DISEASE: EFFECTS ON CARDIOMETABOLIC RISK FACTORS

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Clinical and epidemiological studies provide support that long-chain omega-3 (ω3) fatty acids from fish and fish oils are cardioprotective, particularly in secondary prevention. The two principal ω3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), benefit multiple cardiometabolic risk factors including lipids, blood pressure, vascular reactivity and cardiac function, as well as having antithrombotic, anti-inflammatory and anti-oxidative actions.

ω3 Fatty acids lower blood pressure particularly in hypertensive patients and those with elevated blood pressure. Randomised controlled trials have shown the blood pressure-lowering effects are potentiated by lifestyle changes such as weight loss, sodium restriction and antihypertensive medication. Effects on blood pressure relate to improvements in vascular function, arterial compliance and a cardiac effect mediated by a decrease in heart rate. ω3 Fatty acids reduce triglycerides and VLDL, but have little effect on total cholesterol, HDL-C and LDL-C. They do not significantly affect blood glucose and insulin in normoglycaemic or diabetic individuals. The anti-inflammatory effects of ω3 fatty acids are likely due to attenuating actions on inflammatory eicosanoids, cytokines, endothelial and cell-cell activation, and immune cell function. ω3 Fatty acids are metabolised to potent lipid mediators involved in the resolution of inflammation called resolvins, protectins and maresins. ω3 Fatty acids attenuate oxidative stress and platelet function.

Current recommendations advise an intake of at least two oily fish meals per week (~250-500 mg/day of EPA and DHA) for the general population and in patients with coronary heart disease. ω3 Fatty acid supplementation should be considered as an additional therapy for patients with heart failure and in hypertriglyceridaemic patients. A practical approach for increasing ω3 fatty acid intake is to incorporate fish as part of a healthy diet that includes increased consumption of fruits and vegetables, and moderation of salt intake.

A10 UNIQUE CHANGES IN HEPATIC SPHINGOLIPID SPECIES AFTER HIGH-FAT FEEDING IN BALB/C MICE CORRELATE WITH PROTECTION FROM DIET- INDUCED GLUCOSE INTOLERANCE

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In a recent study examining the response of different mouse strains to high-fat feeding, we showed that C57BL/6, 129X1/SvJ, DBA/2 and FVB/N mice all became glucose intolerant and insulin resistant in response to a high-fat diet, while BALB/c mice were protected from the deterioration in glucose homeostasis and insulin action. Interestingly, all five mouse strains, including BALB/c mice, showed similar changes in many parameters that are commonly associated with glucose intolerance and insulin resistance, such as lipid accumulation in muscle, adipose tissue inflammation and markers of mitochondrial oxidative metabolism in various tissues. What differed in HFD-fed BALB/c mice was a lack of hepatic lipid accumulation.

To explore the full extent of differences in lipid composition in these 5 mouse strains, we have performed lipidomics analysis of liver and skeletal muscle. In muscle, all 5 strains exhibited similar changes in triacylglycerol (TAG), diacylglycerols (DAG), ceramide (Cer) and sphingomyelin (SM) species after high-fat feeding. In contrast, in the liver BALB/c mice were the only strain that did not accumulate any excess TAG and DAG. In addition, the most exciting finding was that the favourable metabolic profile of BALB/c mice was associated with a preferential accumulation of very long-chain Cer and SM species (C>22) over long-chain species (mainly 16:0 and 18:0), while the opposite pattern was observed in the mouse strains that developed diet-induced glucose intolerance and insulin resistance. These sphingolipid differences are likely related to changes in the expression of specific ceramide synthase (CerS) isoforms and the fatty acid elongase Elovl1 in the liver and suggest a potentially important therapeutic role for targeted manipulation of specific sphingolipid species in the treatment of insulin resistance.

A11

F_4-NEUROPROSTANES, NON ENZYMATIC METABOLITES OF DHA ARE RESPONSIBLE OF THE ANTI-ARRHYTMIC PROPERTIES OF DHA

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Since forty years, it is known that polyunsaturated fatty acids (PUFAs) of the n-3 series have cardioprotective effects by preventing cardiac arrhythmias (Bang 1971, Saravan 2010) The main PUFAs involved are eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA). The effects of n-3 PUFAs on cardiac function are still debated, notably because of the lack of information on the mechanisms involved. A diet enriched in n-3 PUFAs (mainly fish-based) is known to lead to enrichment in these fatty acids in cardiac cell membranes. Our hypothesis is that, during an infarct, the generation of reactive oxygen species (ROS) coming from oxidative stress (OS) might be responsible for an oxidation of membrane-bound PUFAs that are highly peroxidable due to the presence of the skipped dienes (Jahn 2008). The oxygenated metabolites thus generated might modulate the activity of ionic channels to exert anti-arrhythmic effects (Jude 2003). We thus decided to investigate the influence of the non-enzymatic peroxidation of DHA and the anti-arrhythmic properties of oxygenated metabolites, to validate if the supposed anti-arrhythmic properties of n-3 PUFAs are not due to their non-enzymatic oxygenated metabolites (Le Guennec PCT/EP2013/075463) We have then *synthesized* oxygenated metabolites of DHA, named neuroprostanes (NeuroPs) (Oger 2008, 2010) and *investigate their anti-arrhythmic effects*.

Thanks to our efficient and flexible strategy, several IsoPs and NeuroPs were successfully synthesized and studied (Galano 2013). The proof of concept was validated both *in vitro* and *in vivo*, showing the anti-arrhythmic properties of some of the non-enzymatic oxygenated metabolites of DHA called NeuroPs. We are still screening IsoPs and NeuroPs and in parallel trying to unravel the signaling pathways likely involved.

Work founded by FRM (DCM2011112326047), INSERM, CNRS, Universities Montpellier 1 and 2.

A12 INVESTIGATION OF STABILITY OF PROTEIN-OLEIC ACID COMPLEXES HAVING ANTI-CANCER ACTIVITY

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Protein-lipid complexes consisting of protein from milk or other selected sources and oleic acid have exhibited broad-spectrum anti-cancer activity not demonstrated by the corresponding apo-protein without oleic acid. These complexes can be produced by incubating heated protein with the lipid (45-85°C, pH 8.3). The thermal denaturation of such protein-lipid complexes was investigated by far-UV circular dichroism and dynamic light scattering, to identify plausible stability characteristics contributing to the anti-cancer activity.

BAMLET (Bovine Alpha-lactalbumin Made LEthal to Tumours), made from alpha-lactalbumin and oleic acid, showed continuous and reversible unfolding as temperature was repeatedly raised and lowered (20°C to 95°C), as did the corresponding apo-protein. BLAGLET (Bovine beta-LActoGlobulin made LEthal to Tumours) also exhibited continuous, reversible unfolding and lack of a sharp transition temperature. The uncomplexed bovine beta-lactoglobulin apo-protein became irreversibly unfolded under the same experimental conditions. There is no published evidence that hen lysozyme and bovine albumin form anti-cancer complexes when bound with oleic acid, and for thermally induced unfolding, the former exhibited a transition temperature whilst the latter did not exhibit complete reversibility. Both alpha-lactalbumin and beta-lactoglobulin prepared with hexadecane instead of oleic acid and not anticipated to have anti-cancer properties, produced samples having size and aggregation state different to those of the protein-oleic acid complexes.

These results suggest that the following stability characteristics may be involved in converting a protein into a BAMLET-like compound possessing anti-cancer activity. 1) Gradual unfolding of the protein rather than a sharp transition temperature, allowing incremental associations of oleic acid with the partially unfolded hydrophobic protein core. 2) In addition to the hydrophobic hydrocarbon tail, the polar head of lipids may be required to stabilise the partially unfolded conformation of BAMLET-like proteins.

A13

FUNCTIONAL LIPIDOMICS: FROM STRUCTURAL CHARACTERIZATION TO REGULATION OF LIPID METABOLIC NETWORKS

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Lipids play essential roles in membrane structure and dynamics, energy homeostasis, and signal transduction. The lipidome of eukaryotic cells comprises several hundred molecular lipid species produced by a metabolic network that interconnects and coordinates the metabolism of fatty acids, glycerophospholipids, glycerolipids, sphingolipids and sterol lipids. One of the central challenges in the field of lipid biology is to understand how cells regulate the dynamics of all lipid metabolic pathways simultaneously, and thereby control the abundance of all lipid species and their impact on cellular processes. Lipidomics methodologies are emerging as new powerful systems biology approaches capable of unraveling the operational principles of global lipid metabolism in health and disease.

For functional studies of lipid metabolism we deploy a lipidomics platform based on automated nanoelectrospray ionization and high-resolution Orbitrap mass spectrometry. This platform support high throughput analysis with high sensitivity, specificity and extensive lipidome coverage. Our platform is complemented by the ALEX software framework which supports automated processing of large lipidomic datasets, implementation of quality control procedures to secure data quality, and rapid and flexible lipidome navigation. The recent implementation of a state-of-the-art Orbitrap Fusion mass spectrometer has extended the analytical capability of the platform supporting both faster analysis, higher mass resolution and multidimensional fragmentation analysis which collectively provide a multitude of complementary spectral datasets for in-depth lipidome characterization. Importantly, our lipidomics platform can be integrated with quantitative proteomic workflows for systems biology studies of the physiological regulation of lipid metabolism.

A14 DEVELOPING NEXT GENERATION TECHNOLOGIES FOR UNMASKING THE LIPIDOME: NEW TOOLS TO ADDRESS THE CHALLENGE OF LIPID ISOMERS

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Significant diversity in the roles of lipids in cell function and dysfunction is becoming increasingly well recognized. At the same time, recent discoveries suggest that the number and structural variety of lipids in nature may also be far greater than previously imagined. Much of this complexity arises from the presence of structurally similar, but functionally distinct, lipid isomers that are not readily distinguished using current analytical technologies. Mass spectrometry is central to all modern lipidomics protocols but it is fundamentally challenged in differentiating lipid isomers because they have a common molecular mass and, oftentimes, share identical tandem mass spectra. This problem is further compounded when multiple isomers are present within the same biological extract. Chromatographic separations, when combined with mass spectrometry, can help in resolving isomeric lipid populations but these can represent a rate-limiting step in the analysis and do not of themselves enable a unique structure to be assigned to each lipid. In our laboratories, we have been developing new workflows based on state-of-the-art mass spectrometry instrumentation to enable efficient separation and unambiguous structure elucidation of isomeric lipids. These methods are designed to help unmask the full molecular complexity of the lipidome.

In this presentation, some of the challenges presented by isomeric lipids in a diversity of lipid extracts ranging from human meibomian gland secretions, to fruit fly pheromones and red blood cell extracts will be discussed. The potential for fast isomer separations in the gas phase using ion-mobility technologies will be presented alongside ion activation technologies that can afford unique structural assignments of lipid isomers. Implications of preliminary findings from these new tools to our understanding of natural lipid structural diversity will be outlined.

A15 **PROFILING OF PHOSPHOLIPID CLASSES USING ^{31}P NMR**

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^{31}P NMR is an effective method for the analysis of phospholipid classes. A major advantage of the method is that it allows for quantitative analysis of phospholipids without the need for calibration using an extensive library of analytical standards. ^{31}P NMR in a sodium cholate detergent system can also give structural information after employing strategies such as pH modification, and deacylation (enzymatic or chemical). Peak position in the NMR spectrum is determined by the environment around the phosphorus atom not by the polarity of the molecule. Therefore, the method is complimentary to chromatographic techniques, and phospholipid classes with similar mobility in TLC (e.g. ethanolamine plasmalogens and phosphatidylethanolamine) can be distinguished and measured by ^{31}P NMR.

The use of ^{31}P NMR for the analysis of phospholipids in a variety of natural products will be discussed. Phospholipids in commercially important products (e.g. dairy and krill phospholipids) are routinely analysed using this method. An advantage of the use of a detergent system for the analysis of dairy ingredients rich in phospholipids (e.g. beta serum powder) is that no extraction step is required prior to analysis.

The discovery and analysis of a number of novel phospholipids found in extremophillic bacteria (e.g. long chain diol phospholipids in Thermomicrobia, and serinol-phospholipids of Verrucomicrobia) will also be presented.

A16 ISOBARIC MASS TAGGING AND TARGETED MS/MS FOR MULTIPLEXED QUANTIFICATION AND CHARACTERIZATION OF AMINOPHOSPHOLIPIDS IN COMPLEX LIPID MIXTURES

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Isobaric mass tagging is widely used for quantitative mass spectrometry in proteomics. This concept is also applicable to lipidomics. However, MS/MS reporter ion overlap from co-isolated precursor ions may compromise the accuracy of quantification for both 'omics' strategies. Moreover, structural information on fatty acyl/alkyl/alkenyl chain identities of quantified lipid ions is not obtained using current tagging methods. Here, in a novel strategy to overcome these limitations, isobaric D_6-'light' and D_6-'heavy' stable isotope containing S,S'-dimethylthiobutanoylhydroxysuccinimide ester iodide (DMBNHS) reagents were used to selectively derivatize PE and PS aminophospholipids present within a crude complex lipid extract from a metastatic colorectal cancer cell line, SW620, and an extract from SW620 cells subjected to siRNA knockdown of the rate limiting peroxisomal enzyme for ether lipid biosynthesis, alkylglyceronephosphate synthase (AGPS). Then, after combining and further derivatization of plasmalogen lipids with iodine/methanol, the samples were subjected to positive ionization mode ultra high resolution ESI-MS, energy resolved CID/HCD-MS/MS, and HCD-MS^3.

Ultra high resolution ESI-MS was used for initial lipid identification at the sum composition level. Then, quantitative analysis of DMBNHS derivatized lipids from each sample was readily achieved following 'targeted' CID- or HCD-MS/MS, by comparing the product ion intensities of the two characteristic neutral loss reporter ions, (i.e., $[M - S(CD_3)_2]^+$ and $[M - S(CH_3)_2]^+$) from the d_6-heavy and d_6-light labelled lipids, even in the presence of co-isolated and co-fragmented lipid precursor ions. HCD-MS^3 of the neutral loss 'reporter ions' then yielded characteristic product ions indicative of lipid acyl, alkyl or alkenyl chain identities, as well as allowing secondary quantification of isobaric mass lipids (e.g., different combinations of acyl chain compositions) that are present at a given precursor ion mass value.

A17 RESOLUTION OF LIPID ISOBARS BY MASS SPECTROMETRY USING DIFFERENTIAL MOBILITY SEPARATION (DMS)

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Lipids mediate diverse physiological processes such as metabolic homeostasis and inflammation and are implicated in the pathophysiology of inflammatory-related diseases. Lipidomics is an established field, but has recently undergone a renaissance as interest in the subject and advances in technology have spurred research aimed at identifying global lipid profile changes in biological samples. The human lipidome contains >100,000 different molecular species found within a small mass range; consequently, isobaric overlap makes unambiguous identification and quantitation of lipid species difficult. Herein, methods that utilize Differential Mobility Spectrometry (DMS) coupled to a triple quadrupole linear ion trap and a time of flight mass spectrometer to isolate lipid classes for MS analysis have been investigated. Infusion- and HPLC-based experiments indicate DMS, using a complex lipid extract, can generate 'clean' MS spectra for individual lipid classes with minimal interference. As a consequence to isolating lipid classes prior to MS analysis, quantitative and qualitative experiments are greatly improved.

The technique of DMS has been applied to a number of difficult analytical challenges in the lipidomics field. For example, eicosanoids, a class of lipids with many isobaric isomers, are readily isolated using DMS, and positional isomers as well as enantiomers can be separated, which greatly reduces the dependence on chromatography to resolve these species. The technique has also been used to elucidate the positions of fatty acids along the glycerol backbone of phospholipids and triglycerides. The most powerful demonstration of the use of DMS in lipidomics analysis is evident during product ion analysis. Due to extensive isobaric overlap within the lipidome there are multiple unexplained fragments in a typical product ion spectrum. However, using DMS to help isolate the precursor ion of interest during MS/MS analyses generates simplified product ion spectra for easier identification and increased quantitative rigor. These data indicate DMS positioned at the entrance to the mass spectrometer significantly simplifies and improves lipidomic analysis by triple quadrupole and accurate instrument mass spectrometry and is a powerful tool to address the complex problem of resolving individual lipid mediators without the need for extensive HPLC method development.

A18 **THE UPS AND DOWNS OF CHOLESTEROL HOMEOSTASIS**

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An emerging theme in the regulation of cholesterol homeostasis is the role of the ubiquitin proteasome system (UPS), through which proteins are ubiquitylated and then degraded in response to specific signals. The UPS controls all aspects of cholesterol metabolism including its synthesis, uptake, and efflux. Our recent work has uncovered the ubiquitylation and degradation of the key players in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and squalene monooxygenase (SM). We have also explored which E3 ligases are involved in the ubiquitylation and subsequent degradation of these enzymes.

A19

A LIPIDOMIC STUDY OF CERAMIDE METABOLISM IN THE LIVER AND ITS ROLE IN TWO LYSOSOMAL LIPIDOSES: DRUG-INDUCED PHOSPHOLIPIDOSIS AND SANDHOFF DISEASE

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Owing to its role in regulating major cellular functions, a lipidomics study was undertaken on lysosomal lipidoses, drug-induced phospholipidosis (DIPL) and Sandhoff disease (SD). Open-profiling of all detectable lipids was achieved using data obtained from ultra-performance chromatography coupled to high-resolution mass spectrometry (UPLC/MS). These data were then pre-processed with and analysed using multivariate statistical analysis. This was followed by targeted analysis of six specific ceramide species using tandem MS using multiple reaction monitoring (MRM). Chloroquine DIPL was studied in Hep G2 cells and liver from rats, whilst SD was assessed in the liver from a mouse model.

Although the total ceramide content was not affected by the lysosomal lipidoses, compared with the controls, the proportions of the different fatty acyl chain lengths in the pool of ceramides were changed, including a common increase in C16:0 ceramide. In the case of DIPL, the change to the composition of the ceramides was also accompanied by an alteration to the expression of the ceramide synthase genes, as measured by qPCR in both intact tissue and Hep G2 cells.

These results demonstrate that ceramides are selectively affected by lysosomal lipidoses in liver according to their fatty acyl chain length, and this is associated with changes in ceramide synthases. These alterations may lead to changes in membrane morphology and affect a variety of cellular functions.

A20 **PROTEIN KINASE C EPSILON DELETION IN ADIPOSE TISSUE, BUT NOT IN LIVER, MODULATES FATTY ACID MOBILISATION AND HEPATIC INSULIN ACTION**

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Hepatic insulin resistance is a key feature of Type 2 Diabetes and has been linked to activation of the lipid-sensitive signalling enzyme protein kinase C epsilon (PKCε). We have previously shown that global deletion of PKCε protects fat-fed mice against glucose intolerance and improves insulin action in the liver. We next generated liver-specific PKCε KO (LEpsKO) mice and subjected them to a high fat diet, but unexpectedly, LEpsKO mice did not exhibit any improvement in glucose tolerance. However, insulin also has indirect effects on hepatic glucose production (HGP), for example through the rapid suppression of fatty acid (FA) release from adipose tissue. Because we have previously observed reduced FA release from adipose tissue in global PKCε KO mice, we next generated adipose tissue-specific PKCε KO (AdEpsKO) mice. When AdEpsKO mice were fed a high fat diet and subjected to ipGTTs after 1, 8 and 16 weeks, they exhibited a significant improvement in glucose tolerance at each time, when compared to "Cre" and “floxed” control mice (iAUC at 8 weeks: 237 vs 506 and 424 mM.min; n=23, 18 and 29; $P<0.001$). This was not associated with changes in body weight or fat mass, nor an increase in insulin levels, consistent with enhanced insulin sensitivity. Importantly, AdEpsKO mice were better able to suppress plasma FA levels during ipGTTs (to 0.13 vs 0.19 and 0.19 mM at 30 min; $P<0.015$), consistent with a role for PKCε in the modulation of liver insulin action through effects on the release of FA from adipose tissue. In preliminary euglycaemic-hyperinsulinaemic clamp studies, insulin suppressed HGP further in AdEpsKO mice compared to "floxed" controls (by 19.6 vs 12.2 mg/kg/min, n=4, $p<0.05$). These studies demonstrate the importance of PKCε in adipose tissue, and while we have not yet ruled out additional mechanisms, such as potential effects on adipokine release, they support a key role for the suppression of FA supply in the regulation of HGP.

A21 PLASMA LIPID PROFILING TO PREDICT CARDIOVASCULAR EVENTS IN TYPE 2 DIABETES

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Introduction: Type 2 diabetes (T2D) is a major risk factor for cardiovascular disease (CVD). However, risk stratification within this group is challenging. While traditional lipid measurements (elevated cholesterol, triglycerides and/or lowered HDL-C) represent useful risk factors for future cardiovascular events (CVE, myocardial infarction, stroke and CVD death), they do not show the full complexity of the altered lipid metabolism associated with T2D or CVD.

Methods: We applied a lipidomic strategy to identify plasma lipids associated with future cardiovascular CVE in patients with T2D. Plasma lipid profiles containing 310 lipids were measured using electrospray-ionisation tandem mass spectrometry on 3779 individuals selected from the ADVANCE study in a case/cohort design. The cohort consisted of T2D patients who had a CVE during the 5-year follow-up (n=689) and T2D patients who did not have a CVE (n=3167). Weighted Cox regression was used to identify lipid species associated with future CVE.

Results: We observed significant associations between 47 lipid species and CVE ($p<0.05$, corrected for multiple comparison using the Benjamini-Hochberg method). Sphingolipids, phospholipids (including lyso- and ether-linked species), cholesteryl esters and glycerolipids were associated with future CVE. Glycosphingolipids showed the strongest positive associations. Interestingly, ether linked phospholipids were both positively and negatively associated while phospholipid and glycerolipid species containing omega-3 and -6 polyunsaturated fatty acids showed negative associations with future CVE.

Conclusion: The strong associations observed between plasma lipids and future CVE suggest that these lipids may represent new therapeutic targets and biomarkers for CVD risk stratification in T2D.

A22 A MORE ROBUST LIPIDOMICS WORKFLOW FOR HIGH-RESOLUTION LC-MS AND DATA DEPENDENT MS-MS USING A HIGH-FIELD ORBITRAP MS AND LIPIDSEARCH SOFTWARE

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The application of lipidomic profiling to disease phenotype analysis is a rapidly growing aspect of translational medical research. Identification of unique lipid biomarkers has the potential to distinguish healthy individuals from individuals at risk for disease, detect diseases earlier, and facilitate development of personalized treatments. Identification of lipids by untargeted lipidomics requires sophisticated software with an extensive lipid database and the mass spectrometer employed must be capable of separating overlapping isobaric and isomeric lipid ions.

Total lipid extracts from bovine heart or liver (Avanti Polar Lipids) were separated via a fused-core C18 column using a Dionex 3000 RSLC binary chromatograph coupled to the mass spectrometer operated in positive or negative ion mode. LC/MS and MS-MS analyses of bovine lipid extracts were performed using a Q Exactive high-field Orbitrap mass spectrometer operating at 120K mass resolution and Top 20 MS^2 and a Q Exactive Plus operated at 70K resolution and Top 15 MS^2. The MS experiments were designed to maximize lipid annotation and the cycle times were 2.4 sec.

The total number of lipid species in each experiment was annotated by LipidSearch software (Thermo Scientific) to provide a relative comparison of performance. New algorithms and features in LipidSearch 4.1 software were implemented to provide more confidence in automated annotation of lipid species in complex samples. Chromatographic artifacts such as poor peak shape and peak tailing are automatically identified and flagged for review; rejected peaks and then are filtered from the results summary thus providing more robust annotation.

The higher mass resolution and speed of the HF instrument provided at least 20% more lipid annotations than the Q Exactive Plus with the same experimental cycle time. Thus, both mass resolution and sufficient speed are very important factors in LC-MS based lipidomics experiments.

A23 **LIPID SYNTHESIS AND STORAGE IN HUMAN METABOLIC DISORDERS**

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Obesity is characterized by accumulation of adipocytes loaded with lipid droplets (LDs). By genetic screening in yeast, we have identified a large number of gene products that regulate the size and number of LDs. In particular, we demonstrate that deletion of a previously uncharacterized gene, *FLD1*, results in the formation of “super-sized” LDs (>50 times the volume of normal ones). Interestingly, null mutations of seipin (the human orthologue of Fld1p), are associated with human Berardinelli-Seip Congenital Lipodystrophy 2 (BSCL2). We use mouse and fly models to confirm an essential role of seipin in adipogenesis and therefore, seipin regulates two important aspects of lipid storage: adipocyte differentiation (systemic lipid storage) and lipid droplet formation (cellular lipid storage). Our recent results suggest that seipin functions in the metabolism of phospholipids, and that seipin deficiency causes accumulation of certain lipid species, such as phosphatidic acid. These accumulated lipids may interfere with PPARgamma function during adipocyte differentiation, causing severe lipodystrophy. These lipid species may also cause morphological changes of LDs, e.g. the formation of “supersized” LDs, in other cell types.

A24 TOWARDS DETERMINATION OF NATURAL VARIATION OF BLOOD LIPIDS IN HEALTHY INDIVIDUALS

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Many unanswered questions remain on the impact of genome natural variation in healthy populations. The basis of biological variation cannot be completely explained by genomics and proteomics. Lipidomics offers an avenue to complement these traditional technologies, studying lipid profiles across various conditions. The recent advances in mass spectrometry complement existing platforms in providing rapid and sensitive methods for lipids profiling.

Lipids have been found to be important in human health as their dysregulation has been reported in pathological conditions. However, it is imperative to understand how lipids vary in normal physiological settings. As such, this study aims to explore the range of biological variation in human plasma of various lipids including glycerophospholipids, sphingolipids and sterol derivatives, in 360 healthy fasting Singaporeans. Targeted mass spectrometry using multiple reaction monitoring (MRM) was used to quantify over 100 individual lipid species. In a first step, variation that is introduced by sample pre-processing and instrumentation, i.e. technical variation, has been carefully considered and the importance of using quality control samples in study design was recognized. Issues involving the analysis of multiple batches by mass spectrometry and batch effect corrections were addressed prior to considering any biological variation.

In this study, we identified 1) within-individual and inter-individual variation in lipid levels 2) ethnicity dependent differences across lipid species, in particular plasmalogen levels. We are currently validating these findings with an additional 113 healthy Australian samples on over 300 lipid species. Ultimately, this work aims to quantify the biological variation of lipids in a representative set of healthy Singaporeans across three ethnicities and a representative set of the Australian population. This will provide baseline data to understand the biological relevance of natural variation in lipids.

A25 LOSS OF NEUROPROTECTIVE SPHINGOSINE 1-PHOSPHATE IN THE EARLY STAGES OF ALZHEIMER'S DISEASE

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Background: The lipid derived signalling metabolite, sphingosine 1-phosphate (S1P), is essential for nervous system, vascular development and integrity. In our study we investigated if alterations to S1P metabolism occur early in the pathogenesis of Alzheimer's disease (AD), and whether S1P protects cultured neurons against the toxicity of amyloid-β oligomers, which are a pathological hallmark of AD. Expression levels of brain derived neurotrophic factor (BDNF), a promoter of synaptic plasticity, memory processes and learning, was also investigated in the presence of S1P to further explore the neuroprotective potential of this essential sphingolipid.
Methods: Liquid chromatography-tandem mass spectrometry was used to measure S1P levels in six brain regions, in a cohort of 34 post-mortem brains, that were differentially affected by AD pathology according to Braak neurofibrillary tangle (NFT) staging. The neuroprotective effect of S1P in the presence of amyloid-β, which is known to be synaptotoxic, was assessed using primary hippocampal neuron cultures and confocal microscopy. The effect of S1P on expression of a panel of neurotrophic factors including BDNF was assessed using quantitative PCR.
Results: S1P levels decreased with NFT development, and a decline in S1P was most pronounced in brain regions where NFT pathology commences early in AD pathogenesis. Loss of S1P was statistically significant in Braak stages III/IV and V/VI for hippocampus and temporal cortex ($P<0.05$). This loss in S1P was associated with a loss of activity of the enzymes sphingosine kinase1 and 2, and hippocampal SphK1 activity was significantly correlated with S1P levels. Exogenous S1P in cultured astrocytes and a neuroblastoma cell line increased expression of BDNF; whilst loss of S1P synthesis reduced BDNF expression. S1P protected cultured hippocampal neurons against disruption to dendritic morphology in the presence of amyloid-β.
Conclusions: AD progression and its associated cognitive decline are accompanied by an early decline in neuroprotective factor S1P. Evidence of S1P in upregulating BDNF expression and protection of synaptic morphology in hippocampal neurons exposed to amyloid-β lend increasing support to the potential of correcting S1P signalling defects in the treatment of neurodegenerative diseases such as AD.

A26

OF MICE, PIGS AND MEN: SHOTGUN-LIPIDOMICS ANALYSIS OF MITOCHONDRIAL PHOSPHOLIPIDS FROM MAMMALS WITH VERY DIFFERENT LIFESPANS

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Maximal lifespan is positively correlated with mammal body mass, with smaller animals having short maximal lifespans (e.g. mice, 4 years) and larger mammals having longer maximal lifespans (e.g. pigs, 27 years). Humans are an exception to this trend, with a comparable body mass to pigs but a notably longer maximal lifespan (122 years). The relative proportion of polyunsaturated fatty acids (PUFA) present in membrane phospholipids is inversely related to body mass, and the peroxidation of these PUFA has been identified as one possible mechanism of aging. It has been proposed that human membranes may contain lower proportions of PUFA than predicted for our body mass, but human data in this area is rare. Here we show that the proportion of PUFA in the total phospholipid content of human muscle, liver and brain mitochondria is lower than the mitochondria of mice and pig tissues. This lower level of PUFA was found in the phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS) fractions of human muscle and liver mitochondria, but only in the PC fraction of brain. The lower level of PUFA in human tissues was found to be due to an higher relative abundance of the most common mammalian phospholipid, PC 16:0_18:1, at the expense of PE and PS species which contained PUFA. Our results support the hypothesis that membrane composition may be a stronger predictor of maximal lifespan than body mass.

Abstracts of Poster Presentations

P1 REGULATION OF ABCG1 LIPID TRANSPORTER BY E3 UBIQUITIN LIGASE ENZYMES

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The ATP-binding cassette transporter, ABCG1, has an essential role in cellular cholesterol homeostasis, and dysregulation has been associated with a number of high burden diseases, such as cardiovascular disease, diabetes and Alzheimer disease. The transporter is highly regulated at the post-translational level through various mechanisms, including ubiquitination and proteasomal degradation. Protein ubiquitination is carried out by a trio of ligases (named E1, E2 and E3) of which the E3 ligases determine the substrate specificity. Using mass spectrometry, we identified a number of E3 ligases that were associated with ABCG1. We then characterised the role of two of these on ABCG1 protein stability using siRNA knockdown experiments in CHOK1 cells overexpressing ABCG1. Single knockdown of each E3 ligase by siRNA significantly increased ABCG1 protein levels. Moreover, double knockdown for both E3 ligases further stabilised ABCG1, suggesting that these two E3 ligases interact with ABCG1 at different parts of the pathway. Consistent with this finding, cholesterol efflux assays showed that the activity of ABCG1 increased after single knockdown of each E3 ligase and further increased after double knockdown, indicating that these E3 ligases are fundamental enzymes in regulating ABCG1 cellular stability and activity. Given the fact that E3 ligases are the rate limiting enzymes in the ubiqitination process, targeting these pathways may provide a novel therapeutic approach to increase the abundance and activity of ABCG1 and thus enhance reverse cholesterol transport.

P2 EFFECT OF ANTIPSYCHOTIC DRUGS ON SPHINGOLIPIDS IN PERIPHERAL METABOLIC TISSUES

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Introduction: Olanzapine (OLAN) and clozapine (CLOZ) are antipsychotic drugs prescribed to treat schizophrenia and bipolar disorder but can cause metabolic side effects, such as hyperglycaemia, insulin-resistance and type II diabetes mellitus (T2DM). Antipsychotic co-treatment with the anti-diabetic drug liraglutide (LIRA) may prevent these metabolic side-effects. Ceramide (Cer) and sphingomyelin (SM) are sphingolipid signalling molecules linked to T2DM by promoting hepatic glucose output (HGO) and insulin-resistance; however, whether diabetogenic antipsychotics effect sphingolipid concentration in key glucometabolic tissues is unknown.

Methods: Adult female rats were acutely administered OLAN (1mg/kg), LIRA (0.4mg/kg), OLAN+LIRA, CLOZ (12mg/kg), CLOZ+LIRA or vehicle (control) (n=6/group), followed by euthanasia 1-hr post-treatment. Total liver, fast (gastrocnemius) and slow-twitch (soleus) skeletal muscle Cer and SM concentrations were measured using tandem mass spectrometry.

Results: Hepatic Cer and SM concentrations decreased in the CLOZ (p<0.01) and OLAN+LIRA groups (p<0.05) compared to controls. CLOZ and OLAN increased soleus SM *vs* controls (p<0.05). CLOZ+LIRA resulted in control levels of soleus SM, while OLAN+LIRA increased soleus SM *vs* controls (p<0.05). Soleus Cer did not change *vs* controls; however, the OLAN and CLOZ groups significantly differed (p<0.01). There was no effect of treatment on gastrocnemius Cer or SM concentration (p>0.05).

Conclusion: These novel findings demonstrate an acute antipsychotic effect on sphingolipid concentration in the liver and slow-twitch, but not fast twitch, skeletal muscle. The antipsychotic increase in soleus SM is consistent with the role of sphingolipids in insulin-resistance and may be implicated in the glucose dysfunction side-effects of these drugs. LIRA co-treatment restored soleus SM levels in the CLOZ but not OLAN groups. Decreased hepatic Cer and SM following CLOZ was unexpected as liver Cer accumulation increases HGO. Overall, these results support a role for sphingolipids in the insulin-resistance side-effects of diabetogenic antipsychotic drugs; however, further investigation is required.

P3 **PLASMA LIPIDOMIC ANALYSIS OF LARGE COHORTS**

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The analysis of cohorts containing hundreds to thousands of plasma samples places specific demands on the lipidomics assay with respect to throughput and long-term reproducibility. Unfortunately, long-term reproducibility is time-consuming and expensive to determine, leading to a paucity of data on the issue. Here we present the methodology currently in use at Baker IDI for performing plasma lipidomics on large sample cohorts with data on its performance.

We use a reverse phase UPLC-MS/MS approach in which we measure in excess of 350 lipids per sample in 10 minutes. We recently completed analysis of ~4000 samples from the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial and ~12,000 samples drawn from the Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) study. To complete analysis across cohorts of this size ultimately requires comparison of samples analysed weeks to months apart. We have adopted an analytical design in which samples were extracted and analysed in batches of 500 (ADVANCE) or 1000 (LIPID) between breaks for instrument cleaning and calibration. Technical variation was assessed by the inclusion of multiple plasma quality control (PQC) samples within each batch and the variation specifically arising from the UPLC-MS/MS was assessed using pooled lipid extracts as technical quality control (TQC) samples.

In the ADVANCE trial we found that across the entire sample set 101 lipids had a CV < 15%. Within batches, greater reproducibility was achieved with 214 lipids having a CV < 15% (median across all batches). In comparison 321 lipids had a CV < 15% in the TQCs indicating significant variation arises from the lipid extraction process. Alignment of the batches to correct for UPLC-MS/MS variation by median centring based on the TQCs significantly improved the reproducibility (assessed using the PQCs) with 167 lipids having a CV < 15% across the entire sample set.

P4 **RAPID AND UNAMBIGUOUS CHARACTERIZATION OF ACYL CHAIN POSITION IN UNSATURATED PHOSPHATIDYLCHOLINES USING DIFFERENTIAL MOBILITY AND MASS SPECTROMETRY**

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Glycerophospholipids (GPs) that differ in the relative position of the two fatty acyl chains on the glycerol backbone (*i.e.*, *sn*-positional isomers) can have distinct physicochemical properties. However the unambiguous assignment of acyl chain position to an individual glycerophospholipid remains a significant analytical challenge. We have developed a workflow where phosphatidylcholines (PCs) are subjected to electrospray ionization in the presence of silver acetate for characterization as $[M + Ag]^+$ adducts using a combination of differential mobility spectrometry and mass spectrometry (DMS-MS). When infused as a mixture, $[PC (16:0/18:1) + Ag]^+$ and $[PC (18:1/16:0) + Ag]^+$ are transmitted through an AB SCIEX SelexION™ DMS cell at discrete compensation voltages prior to MS analysis with an AB SCIEX QTRAP® 5500 triple quadrupole mass spectrometer. Varying the relative amount of these PC synthetic standards allows facile and unambiguous assignment of the *sn*-positions of the fatty acyl chains for each isomer. Integration of the well-resolved ion populations provides a rapid method (< 3 mins) for relative quantification of these lipid isomers. Furthermore tandem mass spectra recorded for each of the $[PC (16:0\_18:1) + Ag]^+$ isomers in isolation reveal that specific product ions alone are not exclusive indicators for acyl chain *sn*-position.

The DMS-MS workflow and two MS-only approaches were used to quantify the relative amount of *sn*-positional isomers in synthetic mixtures; all three methods were benchmarked against established enzymatic approaches. The DMS-MS results not only show excellent agreement with the enzyme assay but also provide superior accuracy over the MS-only workflows. We have demonstrated the advantages of this DMS-MS method in identification and quantification of GP isomer populations by direct analysis of complex biological extracts without any prior fractionation.

P5 CHOLESTEROL METABOLISM IS DISTURBED EARLY IN HUMAN ALZHEIMER'S DISEASE BRAIN

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Cholesterol is a highly abundant lipid in the brain, but there is no transport across the blood brain barrier. Its synthesis and metabolism in the brain is tightly regulated to maintain normal neurological function. Alzheimer's disease (AD) is a major neurodegenerative disease characterised by progressive loss of neurons in the hippocampus that leads to dementia and memory loss. Lipid disturbances and peroxidation are believed to contribute to AD pathophysiology. In order to identify potential biomarkers of AD progression, sterol changes (including cholesterol metabolites and synthetic precursors) were examined in human AD post mortem tissue that was obtained from the Australian Brain Bank Network,

Lipid was extracted from hippocampus (HC) and cerebellum (CB) and sterol levels were analysed using GC-MS/MS and compared to the clinical severity of AD (n= 9 control and n=25 Braak stage I-VI). Total cholesterol levels of both brain regions were no different in AD compared to controls. The major brain cholesterol metabolite 24-hydroxycholesterol (24-OHC) formed by the neuronal specific enzyme CYP46A1, was significantly reduced in HC at later AD stages ($p>0.05$), but was unaffected in CB. At later AD stages in HC, 27-hydroxycholesterol was significant increased, but not in CB. Interestingly, in both brain regions we detected significant decreases in phytosterols (dietary derived) and the cholesterol synthetic precursor 24,25-dihydrolanosterol (DHL) ($p>0.05$).

Our data illustrates that the cholesterol CYP46A1 metabolic pathway is progressively disturbed during AD in HC, where the most severe atrophy occurs. This confirms previous studies of AD patients that detected altered 24-OHC plasma and CSF levels. Likewise, reduced DHL and phytosterols supports previous reports of reduced cholesterol synthetic precursors and phytosterols in CSF and plasma of AD patients. Our results indicate that altered brain sterols are an early pathological event in AD. Elucidating pathophysiological changes in brain cholesterol provides potential biomarkers for examining neurodegenerative disease and offers new targets for therapeutic intervention.

P6 DOCOSAHEXAENOIC AND ARACHIDONIC ACID PEROXIDATION: IT'S A WITHIN MOLECULE CASCADE

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The oxidation of phospholipids i.e. peroxidation is a well-known natural phenomenon associated with both health and disease. We sought to compare the peroxidation kinetics of phospholipids with different unsaturation levels (i.e. 18:0, 18:1n-9, 18:2n-6, 20:4n-6 and 22:6n-3 at the sn-2 and 16:0 at sn-1 position). Peroxidation was initiated by ferrous iron and performed on phosphatidylcholine (PC) molecules either free in solution or formed into liposomes. Fatty acid levels, oxygen consumption plus lipid hydroperoxide and malondialdehyde production were measured from the same incubations, at the same time during periods of maximal elicitable peroxidation. PCs with highly peroxidisable fatty acids i.e. PC 20:4n-6 and PC 22:6n-3, in the same incubation tended to be either fully peroxidise (as measured by oxygen consumption) in proportion to their number of bisallylic groups (i.e. 3 and 5 respectively) or remained intact. Rates of peroxidation of PCs with multiple bisallylic groups (i.e. 20:4n-6 and 22:6n-3) peroxidised at 2-3 times the rate per bisallylic bond than the same phospholipid with 18:2n-6.

These results suggest that propagation of peroxidation (H-atom transfer) is firstly an intramolecular process that is several-fold faster than intermolecular peroxidation. It was found that PCs in solution peroxidised twice as fast as those in liposomes suggesting only half of the phospholipids in liposomes were available to peroxidise i.e. the outer leaflet. The experiments suggest that even after heavy peroxidation of the outer leaflet, the inner leaflet of liposomes is unaffected and might indicate how cells protect themselves from external peroxidation and maintain control over internal peroxidation. Intramolecular peroxidation is likely to produce highly localized sites of peroxidation product that together with internal control of peroxidation of the inner leaflet of membranes provides new insight into how cells control peroxidation at the membrane level.

P7 DOES DIETARY INTERVENTION CHANGE URINE ISOPROSTANE LEVELS IN PROSTATE CANCER PATIENTS? - A PILOT STUDY.

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Death from prostate cancer (CaP) ranks fourth in Australian male death from any cause, with 2924 men dying from CaP in 2011[1]. Nonetheless, improvement in disease-free survival and reduction in overall mortality has been achieved through better diagnosis, monitoring and treatment, albeit with associated increased health costs. Despite this success, mortality and cost have led to a growing search for improved prevention, diagnosis, monitoring and treatment strategies. The essential dietary fatty acid arachidonic acid (AA), its oxidative metabolites and the biochemical pathways that generate them are now known "epidrivers" of CaP[2]. Thus, changes in lipid profiles of AA metabolites in urine may provide an avenue to rapidly and non-invasively monitor the effect of CaP therapy and may be a simple marker to follow the effect of preventative strategies such as dietary modification. Here we describe our efforts to establish, validate and utilise an LC-MS/MS assay to monitor urinary isoprostanes in a pilot study of a six-week dietary intervention with phytochemical-supplemented cereal in patients with prostate cancer.

1. AIHW 2013. Prostate cancer in Australia. Cancer series 79. Cat. no. CAN 76. Canberra: AIHW. 2. Scott., K. F et al. Biochimie, (2010) 92:601-610.

P8 ALTERED CHOLESTEROL METABOLISM AND INCREASED CHOLESTEROL PEROXIDATION IN HUMAN POST-MORTEM HUNTINGTON'S DISEASE BRAIN

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The brain is highly abundant in lipid (~50% dry weight), and contains the highest concentration of cholesterol in the body. Disturbances of lipid pathways and lipid peroxidation have been identified as important factors in the pathophysiology of many neurodegenerative diseases, including Huntington's disease (HD). HD is a progressive neurodegenerative disease caused by a polyglutamine mutation in the Huntingtin protein and is characterised by severe atrophy in the striatum as well as pathophysiology of the cortex.

This study aimed to measure changes in cholesterol metabolites and cholesterol oxidation products (COPs - lipid damage biomarkers), in human HD post mortem tissue and identify potential biomarkers of the disease. Lipid was extracted from post mortem brain tissue (obtained from the ABBN) and analysed using GC-MS/MS. Five brain regions of HD (≥ stage 3) and age matched controls (n=9 per group) were analysed; striatum (putamen and caudate), cortex (white and grey matter) and cerebellum, which is not considered to experience HD pathology

The major brain cholesterol metabolite 24-hydroxycholesterol (24-OHC) was significantly reduced in HD striatum and cortex, but was unaffected in CB. 27-hydroxycholesterol, a minor metabolite, was significantly elevated in HD striatum and cortex, but was unaffected in CB. 7-Ketocholesterol (COP) was significantly increased in HD striatum with small non-significant increases in 7β-OH cholesterol (COP). No significant difference was detected by GC-MS/MS in levels of the lipid peroxidation biomarker F_2-Isoprostanes nor total cholesterol, for any brain region.

Our data illustrates that the major cholesterol CYP46A1 metabolic pathway is disturbed in HD brain, particularly the striatum where the most severe atrophy occurs. Oxidative stress is also evident in multiple HD brain regions, particularly putamen. Elucidating pathophysiological changes in brain sterols provides potential biomarkers for examining neurodegenerative disease and offers new targets for therapeutic intervention.

P9 MAJOR ENZYMES OF CHOLESTEROL SYNTHESIS AND METABOLISM ARE DISTURBED IN HUMAN HUNTINGTON'S DISEASE BRAIN

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Cholesterol is a highly abundant lipid in the brain. Its synthesis and metabolism is tightly regulated to maintain normal neurological function, but sterol pathways are known to be disturbed in multiple neurodegenerative diseases. Huntington's disease (HD) is a heritable neurodegenerative disease characterised by severe atrophy and neuron loss in the striatum. This study aimed to examine changes in key enzymes and sterols of cholesterol metabolism and synthesis in post mortem striatum from HD cases.

Post mortem striatum (putamen obtained from the ABBN) from HD (≥ stage 3) and aged matched controls were analysed (n=9 per group). Protein levels of cholesterol pathway enzymes were assessed by Western Blotting and mRNA expression was analysed by quantitative real-time PCR. Cholesterol metabolites and synthetic precursors were measured using GC-MS/MS.
Protein levels and mRNA expression of cholesterol 24-hydroxylase (CYP46A1) were significantly reduced in HD putamen, that was accompanied by a reduced level of its oxysterol product, 24-hydroxycholesterol ($p<0.01$). 24-Dehydrocholesterol reductase (DHCR24 or Seladin-1), an enzyme in the late stage of the cholesterol synthetic pathway, showed reduced protein levels in HD putamen that corresponded with significantly increased levels of its main substrate, desmosterol ($p<0.01$). Total striatal cholesterol levels remained unaffected by HD.

Our data illustrates the major cholesterol metabolic pathway in brain, catalysed by the neuronal specific enzyme CYP46A1 is reduced in HD striatum. This is in line with reports of reduced levels of 24-OH cholesterol in HD patient plasma. The cholesterol synthetic pathway is also perturbed with significant reductions in DHCR24 and increased desmosterol levels. Further studies are examining whether sterol changes in HD striatum may have direct effects on neurons and HD pathophysiology and/or are biomarkers of neuronal loss during HD progression.

P10 POTENTIAL ROLE OF NON-ENZYMATIC OXIDIZED LIPID PRODUCTS OF ADRENIC ACID *IN VIVO*

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Adrenic acid (AdA) is an elongated form of arachidonic acid (AA) in the omega-6 polyunsaturated fatty acids series. It is concentrated in myelin within the white brain matter of primates. AdA is susceptible to free radicals attack to form 7-, 10-, 14- and 17-series of F_2-dihomo-Isoprostanes. Under increased oxygen tension, AdA can also release furan forms, dihomo-Isofurans via different metabolic pathways to generate two classes, which are alkenyl-Isofurans and enediol-Isofurans. Aside for its existence in the brain, very little is known in other biological samples.

In this study, we displayed the presence of F_2-dihomo-Isoprostanes (7(*RS*)-7-F_{2t}-dihomo-isoprostane and 17(*RS*)-17-F_{2t}-dihomo-isoprostane) and dihomo-isofurans (7(*RS*)-ST-Δ^8-11-dihomo-isofuran and 10-*epi*-17(*RS*)-SC-Δ^{15}-11-dihomo-isofuran) in various biological tissues and marine fish body muscles. These levels were compared with products from AA and docosahexaenoic acid (DHA) mainly 5-F_{2t}-isoprostane and 15-F_{2t}-isoprostane, and 10-F_{4t}-neuroprostane and 4(*RS*)-4-F_{4t}-neuroprostane respectively. The lipid component was extracted and analysed using liquid chromatography tandem mass spectrometry.

Analysis of prefrontal cortex of pig brain sample showed the dominance of 4(*RS*)-4-F_{4t}-neuroprostane of the isoprostanoids and 10-*epi*-17(*RS*)-SC-Δ^{15}-11-dihomo-isofuran of the isofuranoids. However, in brain sample of Sprague Dawley rats, 5-F_{2t}-isoprostane and 4(*RS*)-4-F_{4t}-neuroprostane of the isoprostanoids, and 7(*RS*)-ST-Δ^8-11-dihomo-isofuran and 10-*epi*-17(*RS*)-SC-Δ^{15}-11-dihomo-isofuran of the isofuranoids were predominant. In order to see if hyperoxia condition indeed alters the isofuranoids concentration, we exposed excess oxygen to marine medaka fish for 1 and 6 hours. Only 10-*epi*-17(*RS*)-SC-Δ^{15}-11-dihomo-isofuran increased after 6 hours; this elevation was also found to increase sexual behaviour and associated to pheromone levels in male fish.

The presence of adrenic acid endogenously is very low *in vivo.* Despite this, non-enzymatic oxidised lipid products of AdA appear to have a vital role in the systemic metabolism.

P11 A LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD FOR COMPREHENSIVE IDENTIFICATION AND QUANTIFICATION OF POLAR LIPIDS IN MILK

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Milk polar lipids (PLs), including phospholipids and sphingolipids play a major role in the stability of the emulsion status of the milk. Besides nutritional value, PLs in particular sphingolipids have other beneficial effects on human health, such as anti-inflammatory activity, reducing the risk of cardiovascular diseases, reducing cholesterol absorption and so on.

A new LC-MS method for comprehensive identification and quantification of polar lipids in milk with a simple sample pretreatment procedure will be presented. The method enables a more comprehensive characterization of polar lipids compared to other platforms used so far. The method is also robust for relative quantification of all essential polar lipid species found in cow milk, whereas current methods for polar lipid quantification (mostly based on LC-ELSD) can only determine the total amount of each polar lipid class.

The lipid fraction was extracted by a mix of chloroform and methanol and the extract was directly used for PL identification and quantification. PLs were separated by hydrophilic interaction liquid chromatography (HILIC) and detected by an orbitrap mass analyser in positive mode. The structure of PLs was established or confirmed by tandem MS in both positive and negative modes. The method is sensitive (with a LOD for all PL classes ≤ 0.1 ng) and reproducible, enabling simultaneous quantification of 70 PL species within a run of 45 min.

Application of this method allowed us to reveal the strong correlation between the major polar lipid classes, the relative abundance of PL species within each class, and the influence of the almond supplemented diet on the PL profile of milk.

P12 THE INFLUENCE OF MEMBRANE LIPID COMPOSITION, PEROXIDATION AND METABOLISM ON AGEING IN HONEY BEES (*APIS MELLIFERA*).

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The 'Oxidative Stress Theory of Ageing' currently forms the basis of the most accepted mechanistic explanation of ageing and lifespan. It involves interplay between membrane lipid composition and metabolic rate, where the accumulation of oxidative damage is the main cause of ageing, and lifespan is determined by the rate at which this damage occurs. Free radicals are highly reactive molecules produced by mitochondria that can react with oxygen and damage DNA, proteins and lipids. Lipids-radical reactions are particular damaging as they propagate the process (peroxidation) with some fatty acids more capable than others. This suggests that fatty acid composition is actively involved in ageing and it has been proposed that fatty acid composition provides a mechanistic explanation for variation in lifespan among animal species.

This project will investigate the interaction of metabolic rate, membrane fatty acid composition and lipid peroxidation on the lifespan of different phenotypes of honeybees (*Apis mellifera*).

This project will comprise of three studies. Study one and two will examine differences the lipid composition of different honeybee phenotypes (i.e. phenotypes are genetically identical with worker bees living for 5-7 weeks while queen bees live up to 8 years) maintained under wild-type conditions at different ages. The third study will test the influence of lipid composition on bees using diets of different fatty acid composition to test for extension of the lifespan of worker bees.

This research will inform us about what influences ageing by using direct experimentation. Most ageing research has focused on short-living species 'forcing' animal to live-longer through different interventions in highly controlled conditions. The current experiments will investigate a species that naturally maintains a lifespan difference of up to 100-fold under natural conditions.

P13 DEVELOPMENT OF A UHPLC-MS METHOD FOR HIGH THROUGHPUT PLASMA LIPIDOMICS OF LARGE COHORTS

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Targeted lipidomic analysis of human plasma by liquid chromatography mass spectrometry (LC-MS) is providing useful insight into, and potential biomarkers for, a range of disease states. The analysis of large cohorts requires balancing demands of analytical performance such as linear response and reproducibility with practical considerations such as method robustness, throughput and cost. Here we present data on the optimization of several methodological parameters for the plasma lipid profiling methodology used in the analysis of large cohorts at Baker IDI.

We have examined the linear response of multiple lipid classes including sphingolipids phospholipids, glycerolipids and cholesteryl esters, across the physiologically relevant range for human plasma. Specifically, we have examined two solvent systems based on IPA/ACN/H_2O and THF/MeOH/H_20 finding that while the former may have had some advantages, significant problems were encountered with linear response at concentrations relevant for the analysis of plasma samples. To achieve a linear response for the IPA/ACN/H20 system required a reduction in the concentration of the lipid extract to the point where detection of less abundant lipids was no longer practical. Our studies indicate that linearity across the full range of the human plasma lipidome is more readily achieved using a THF based solvent system.

Additionally, we have considered a number of gradient conditions to try and increase throughput. While faster methods increase sample throughput we find that for the ~400 scheduled MRMs examined here ultimately lead to unacceptably short dwell times for methods less than 10 min per sample. Optimisation of the method allowed for MRM windows of at least 42 seconds which is sufficient to include any retention time drift over more than 1000 samples, which results in dwell times greater than 5ms.

In summary, we have developed a robust LC-MS method which achieves a throughput of 130 samples per day, while achieving reasonable performance with respect to linearity and reproducibility, with over 380 features giving a %CV of less than 20% over 50 replicate injections.

P14 BRAIN CHOLESTEROL OXIDATION AND SYNTHESIS IN THE R6/1 MOUSE MODEL OF HUNTINGTON'S DISEASE

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The brain is highly abundant in lipid (~50% dry weight), and contains the highest concentration of cholesterol in the body. Disturbances of lipid pathways and lipid peroxidation have been identified as important factors in the pathophysiology of many neurodegenerative diseases, including Huntington's disease (HD). However, many of the mechanisms and pathways have not been fully examined. HD is a progressive neurodegenerative disease caused by a polyglutamine expansion in the Huntingtin protein and is characterised by severe atrophy in the striatum.

The purpose of this study was to examine changes in precursors, metabolites and cholesterol oxidation products in transgenic HD mouse brain in order to assess their potential as biomarkers of neurodegeneration. A GC-MS/MS method was established to measure multiple sterol metabolites, synthetic precursors and oxidation products as well as F_2-Isoprostanes that were extracted from biological tissue using solid phase extraction. Male and female R6/1 mice and wild type littermates (n=10-11) were sacrificed at 12, 20 and 24 weeks and lipid was extracted from cortex and striatum..

Motor coordination was monitored every 2 weeks using RotaRod apparatus and declined progressively in both sexes of R6/1 mice from 8 weeks of age. Total cholesterol levels were unaffected, but synthetic precursors, lathosterol, lanosterol and zymosterol were all significantly reduced in R6/1 cortex and striatum. The oxidation products 7-ketocholesterol and 7β-hydroxycholesterol were also significantly increased in male R6/1 cortex. In contrast F_2-Isoprostanes (lipid peroxidation biomarker) were not significantly increased in either brain region.
The GC-MS method described provides sensitive analysis of brain cholesterol pathways and reveals a significant defect in cholesterol synthesis and evidence of oxidative stress in HD mouse brain.

P15 TOTAL LIPID, SPECIFICALLY TRIACYLGLYCEROLS, CHOLESTERYL ESTERS AND PHOSPHOTIDYLCHOLINE, ARE INCREASED IN PLACENTAL TISSUE FROM WOMEN WITH PRE-ECLAMPSIA COMPARED TO HEALTHY PLACENTA TISSUE

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The placenta appears to be the central organ of dysfunction in women with pre-eclampsia (PE) and evidence suggests that altered lipid metabolism may play a role in the development of PE. We have previously shown metabolic characteristics associated with liver fat accumulation in PE and hypothesized that placenta may also contain ectopic fat in PE (Mackay et al. 2012). The objective was to obtain a lipid profile of PE and intrauterine growth restriction (IUGR) placenta tissue and compare with the placental lipid profile in healthy pregnancies. Quantitative lipidomics was performed on placental samples. Lipids were extracted from placental tissue into methyl-tert-butyl ether after the addition internal standards. The extracts were analyzed by nano-electrospray mass spectrometry utilising targeted precursor-ion and neutral-loss scans. Approximately 120 individual lipid species were quantified by comparison to the internal standards. Total placenta lipid was calculated as the sum of ceramides, sphingomyelin, cholesteryl esters, triacylglycerols (TAG) and phospholipids. Total placental lipid did not significantly differ between controls (n=68) and IUGR(n=10). Total mean (SEM) placental lipid in women with PE (n=19) was 6417 (303) nmol/g; in women with IUGR was 5562 (449) nmol/g and controls was 5234 (147) nmol/g, hence women with PE had a 22% significantly higher placental lipid compared with controls p=0.0021. This increased lipid in PE compared to controls were due to increased phosphatidylcholine (3606 vs 2896 nmol/g), triacylglycerols (364 vs 268 nmol/g) and cholesteryl esters (404 vs 314 nmol/g, all p<0.001), with approximately half the PC species, nearly all CE species (except 18:2 and 20:5) and most TAG species being higher in PE compared to controls. In conclusion placental tissue from women with PE had increased phosphatidylcholine and storage lipids compared to controls. These data support the hypothesis that placenta in PE is a site of storage of ectopic fat.

Ref: Mackay V et al. Preeclampsia Is Associated With Compromised Maternal Synthesis of Long-Chain Polyunsaturated Fatty Acids, Leading to Offspring Deficiency. Hypertension 2012; 60: 1078-1085

P16 WOMEN WITH PRE-ECLAMPSIA HAVE INCREASED PLACENTAL LIPID BUT THEIR INCREASED VERY LOW DENSITY LIPOPROTEIN PARTICLES IS NOT ASSOCIATED WITH ALTERED LIPID AND FATTY ACID COMPOSITION

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Women with pre-eclampsia (PE) have increased plasma very low density lipoprotein (VLDL) compared to healthy pregnancies. Women with PE and intrauterine growth restriction (IUGR) had 23% to 60% lower polyunsaturated fatty acids in erythrocyte membranes compared with controls (Mackay et al 2012) but the composition of the fatty acid supply within VLDL is unknown. The objective was to determine maternal VLDL lipid, apo B and fatty acid composition and placental lipid concentration in women with PE. Maternal VLDL was isolated from third trimester fasted plasma samples taken from women with PE (n=16), intrauterine growth restriction (IUGR; n=5), and healthy controls (CON; n=45). VLDL triacylglycerol (TAG), total cholesterol (TC), apolipoprotein B (Apo B) and all fatty acids were measured. Placental samples (PE n=19, IUGR n=12, CON n=70) were also taken at birth and total lipid concentration was measured using mass spectrometry. Maternal VLDL apo B (i.e. VLDL particle number) was increased by 73% in PE compared to CON (P<0.001) and IUGR (P=0.031). However VLDL TAG, TC, and FA concentrations per VLDL particle did not differ between groups. Total placental lipid was increased in women with PE by 22% compared with CON (P<0.001). Total placental lipid did not differ between CON and IUGR. In conclusion, women with PE produced nearly double the number of VLDL particles with no change in lipid or fatty acid composition, suggesting no deficiency in maternal liver PUFA synthesis. Increased placental lipid concentration may play a role in the pathogenesis of pre-eclampsia.

Reference:
Mackay V et al. Preeclampsia Is Associated With Compromised Maternal Synthesis of Long-Chain Polyunsaturated Fatty Acids, Leading to Offspring Deficiency. Hypertension 2012; 60: 1078-1085

P17 PLASMA LIPIDS: ASSOCIATION WITH ALCOHOLIC LIVER DISEASE AND POTENTIAL BIOMARKER

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Introduction: Excessive alcohol intake may result in a severe alcoholic liver disease including fibrosis and cirrhosis. Early identification of liver injury in alcoholic liver disease is vital to develop suitable intervention. Given that the initial dysfunction of the liver will result in abnormal lipid metabolism, we have applied lipid profiling technology to characterise and compare plasma lipid profiles from alcoholics without liver cirrhosis (AwLC) to those with advanced alcoholic liver cirrhosis (ALC).

Methods: In a cohort of 59 excessive drinkers (31 ALC and 28 AwLC), we used electrospray ionisation tandem mass spectrometry to measure 335 individual lipid species in plasma. Statistical analysis was performed using logistic regression adjusting for covariates. Multivariate classification models were developed using support vector machines.

Results: Out of 25 lipid classes and subclasses, dihexosylceramide, trihexosylceramide, alkylphosphatidylcholine, lysoalkylphosphatidylcholine, phosphatidylinositol and free cholesterol were significantly associated with ALC after corrections for multiple comparisons ($p < 0.05$, Benjamini Hochberg corrected). Multivariate classification models created with traditional risk factors resulted in an AUC of 0.847 and an accuracy of 79.7%. The addition of lipid measurements to the traditional risk factors improved the classification performance (AUC = 0.892; Accuracy = 81.8%; Net Reclassification Index = 4.11%).

Conclusion: The gain in AUC and accuracy of the combined models demonstrate the potential of plasma lipids as predictors of liver injury in alcoholic liver cirrhosis.

P18 INVESTIGATING CHANGES TO PHOSPHOLIPIDS OF THE HUMAN BRAIN DURING NORMAL AGEING

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Phospholipids make up a substantial portion of the human brain and changes to them are thought to play a role in the pathogenesis of age-related neurodegenerative diseases. Nevertheless, little is known about the changes that phospholipids undergo during the normal ageing process. Therefore the purpose of this study was to examine the changes occurring in the phosphatidylchlolines (PC), phosphatidylethanolamines (PE) and phosphatidylserines (PS) of the human prefrontal cortex (PFC), motor cortex, entorhinal cortex, hippocampus and cerebellum during normal ageing. Post mortem brain tissue was obtained from 36 neurologically normal human brains aged 18 to 104 years. Mitochondrial and microsomal membranes were isolated from each region using differential centrifugation, and phospholipids were extracted using a modified Folch method. Phospholipids were analysed using nano-electrospray ionisation mass spectrometry, and linear regression with age was performed.

Overall, the cerebellum reported the most numerous changes to its phospholipids with age in the mitochondrial and microsomal membranes, while the motor cortex was the least affected by age. The PFC and cerebellum displayed similar age-related changes in both membrane fractions, with large increases seen in both regions for mitochondrial and microsomal PS 18:0_22:6. Large increases with age were also observed in mitochondrial and microsomal PE 18:0_22:6 in the cerebellum only. The largest decreases with age were reported for mitochondrial PS 18:0_18:1 in both the PFC and cerebellum, and in the cerebellum only for microsomal PS 18:0_18:1. PE 18:0_20:4 also decreased with age in the mitochondrial membranes of the PFC, cerebellum and motor cortex, and in the microsomal membranes of the cerebellum and motor cortex. The hippocampus and entorhinal cortex also underwent many small but significant changes with age in phospholipid composition.

While previous studies have shown losses of phospholipids containing 20:4 fatty acids with age in the human brain, this is the first report of phospholipids containing 22:6 fatty acids increasing with age in the brain. This increase in 22:6 with age challenges the findings of earlier studies, and may provide new insight into our understanding of ageing.

P19 DNA SEQUENCING AND LIPID FINGERPRINTING USING TANDEM MASS SPECTROMETRY AS COMPLEMENTARY TECHNIQUES FOR THE CHARACTERISATION OF FUNGAL COLONIES ON POLYMER COATED BUILDING MATERIALS

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COLORBOND® Steel is a polymer coated steel product and a popular roofing material for home-owners in Australia. In most Australian environments, however, melanised fungal colonies develop over time upon the roof's surface. These colonies not only cause unsightly black staining, but also absorb incident infrared radiation, decreasing the thermal efficiency of the roof.

The culpable fungal colonies show morphological similarities, suggesting that the infestation may be attributed to a small range of organisms. Identification of the organisms involved could be crucial to the development of preventative or remediative strategies, specifically tailored to combat this problem. Thus, colonies were taken from COLORBOND® Steel panels mounted at a test site susceptible to infestation and DNA sequencing of these colonies was performed. Due to the limited DNA sequence databases and difficulty in laboratory culturing, however, the assignment of the colonies is limited to higher taxa, e.g. Order or Family only. For this reason, lipid fingerprinting using tandem mass spectrometry was employed as a complementary identification technique.

Lipid fingerprints of laboratory cultured samples of organisms identified as possible DNA matches were compared to lipid fingerprints of colonies from test panels. These complementary analytical approaches supported the identification of several species and allowed other possible DNA matches to be disregarded. In summary, these techniques form a powerful tool to refine the identification of organisms of interest with a high degree of confidence.

P20 EVALUATION OF A LIPID PROFILING SYSTEM USING REVERSE-PHASE LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY AND AUTOMATED LIPID IDENTIFICATION SOFTWARE

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A lipid is generally considered to be any molecule that is insoluble in water and soluble in organic solvents. Biological lipids usually refer to a broad grouping of naturally occurring molecules which includes fatty acids, waxes, eicosanoids, monoglycerides, diglycerides, triglycerides, phospholipids, sphingolipids, sterols, terpenes, prenols, fat-soluble vitamins (such as vitamins A, D, E and K) and others.

The main biological functions of lipids include their central role in energy storage, as structural components of cell membranes, and as important signaling molecules

Mass spectrometry is used frequently for lipidomics, but is not currently in general use at UNSW. As a facility we frequently face problems such as inexperienced instrument users, poor sample preparation practices and a lack of understanding of the experiments involved.

Technical advances in instrumentation (UHPLC and Orbitrap mass analysers) and in software for qualitative and quantitative lipidomics have provided us with a potential 'turnkey' system with robust characteristics and production of high quality data. This type of approach cannot replace dedicated lipidomics laboratories and specialists, but it is hoped that it will provide a general lipidomics solution in a facility environment.

Initial results and evaluations of the system combining LipidSearch software with a Q-Exactive Plus mass spectrometer and C18 UHPLC are presented and discussed.

P21 BIOLOGICAL SAMPLE PREPARATION FOR LC-ESI-MS/MS LIPIDOMICS

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Liquid chromatography coupled with electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) is the predominant analytical method for targeted lipidomics. For lipidomic data to be useful it must necessarily be quantitative mandating the inclusion of stable isotope labelled internal standards into the biological sample *a priori*. However, the multiple reaction-monitoring mode of a triple quadrupole MS affords the ability to simultaneously measure hundreds of lipids prohibiting the ideal isotopologue matching internal standard scenario. Commonly a judicious choice of a single internal standard is employed for each lipid class. Although the introduction of high pressure LC with reverse phase has enabled both polar and non-polar lipids to be separated prior to MS analysis, the method of lipid extraction is critical for the generation of reproducible, quantitative high quality data. Factors to consider include the recovery of endogenous lipids from the sample, the behaviour of the internal standards through the extraction procedure compared with their endogenous counterparts, matrix components causing ion suppression/enhancement in the ESI source, compatibility with further LC separation, and lastly the efficiency of the method in terms of throughput, cost and labour. Traditionally, bi-phasic strategies that extract the polar and non-polar lipids into two-phases consisting of chloroform/methanol and water have been the gold standard. While such methods allow good recoveries of a large suite of lipids, particularly in the organic phase and are effective at removing salts, some lipids namely complex glycosphingolipids, will partition primarily into the aqueous phase thereby requiring additional chromatography. Here we evaluated a simple, high-throughput, single phase extraction method for the measurement of a variety of sphingolipids, complex glycosphingolipids, phospholipids, cholesterol and cholesterol esters from both plasma and tissue homogenates using LC-ESI-MS/MS.

P22 COULD ARACHIDONIC ACID METABOLIZING ENZYMES REGULATE CANCER PROGRESSION THROUGH INTERACTION WITH VIMENTIN?

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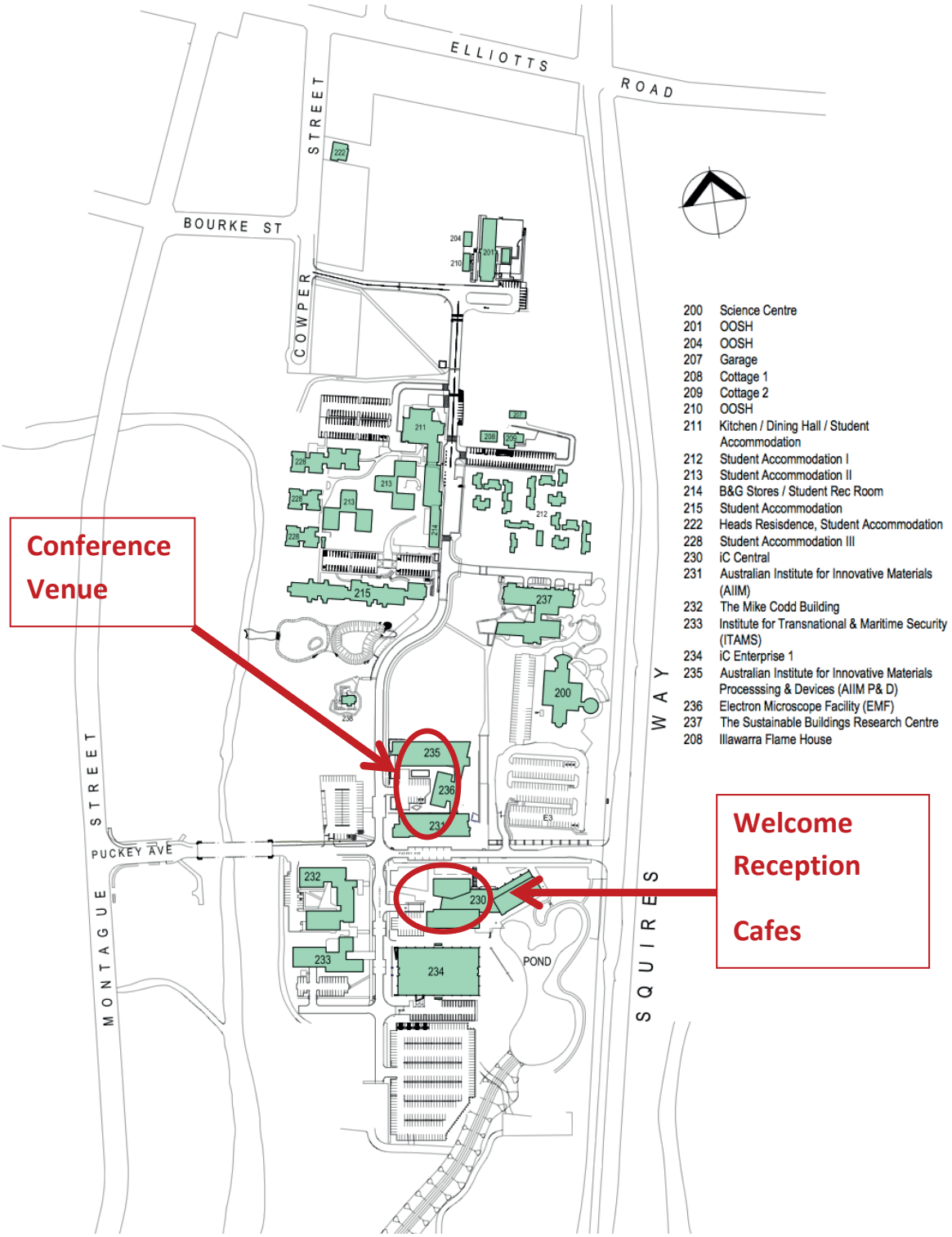
Progression of epithelial cancers is associated with and proposed to be dependent upon a dedifferentiation event, termed EMT, whereby tumour cells transition from a localised epithelial phenotype to a more mobile and invasive mesenchymal phenotype. A defining marker of this transition is the induction of the intermediate filament protein vimentin, often expressed in tumours. We have shown in human primary mesenchymal cells (synovial fibroblasts) that a secreted phospholipase A_2 ($sPLA_2$), hGIIA, known to regulate arachidonic acid metabolism by both catalysis-dependent and –independent mechanisms, is rapidly internalised by these cells and colocalizes with vimentin on exogenous addition. Localisation is blocked by $sPLA_2$ inhibitors that also block hGIIA function[1]. We have also shown that hGIIA, aberrantly expressed in 85% of prostate tumours, stimulates proliferation of prostate cancer cells in culture on exogenous addition, that is blocked by our cyclic peptide hGIIA inhibitors[2] and that hGIIA is also rapidly internalised in these cells. The inhibitors slow the growth of tumours in a xenograft model of prostate cancer on oral administration. Taken together, these data raise the possibility that hGIIA function in prostate cancer cells may be mediated at least in part through interaction with vimentin.

1. Lee, L.K. *et al*. J. Biol. Chem. (2013) 288: 15269-15279. 2. Sved, et al. Cancer Res. (2004) 64, 6934–6940.

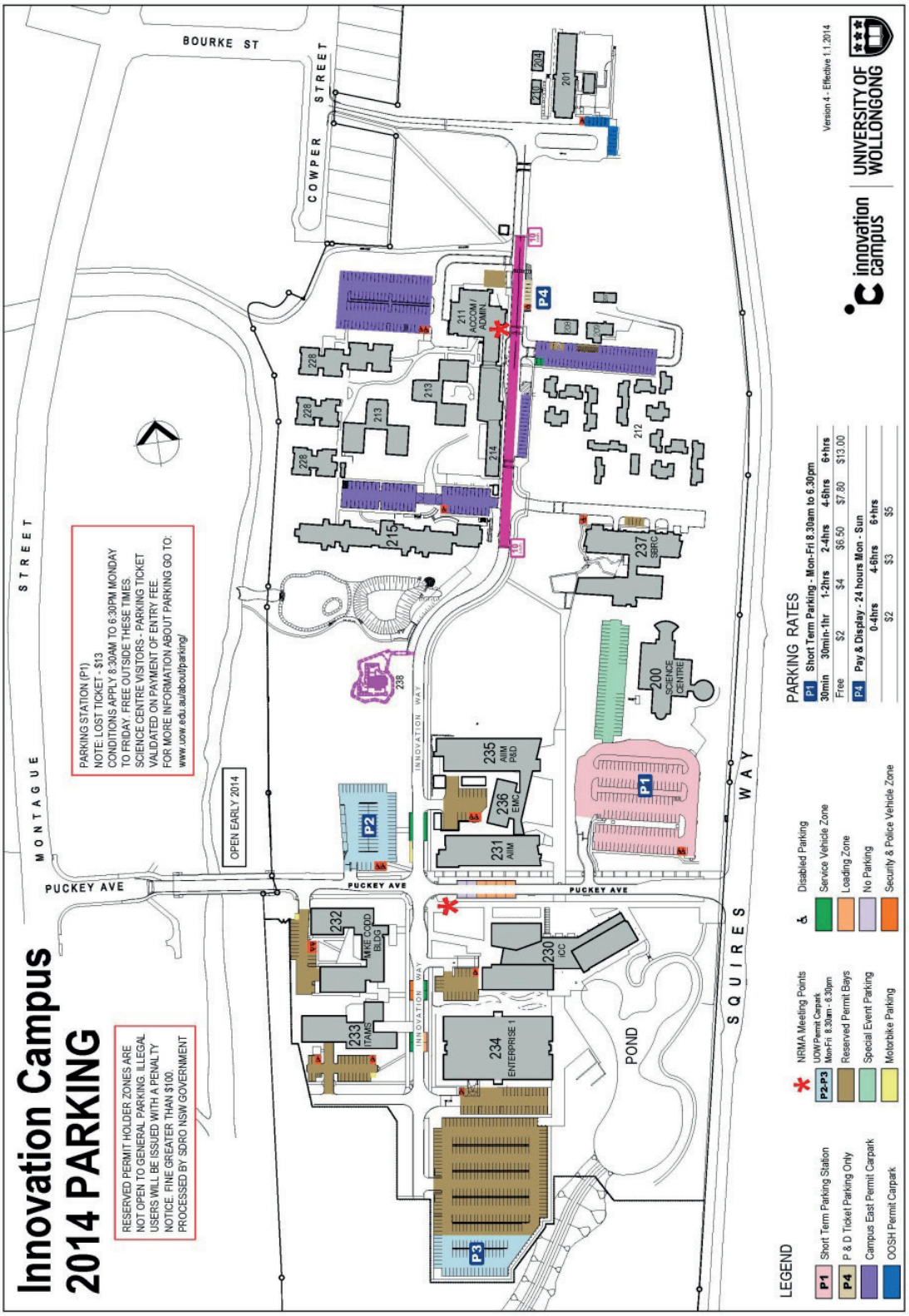
Maps

Map 1 - Innovation Campus and Wollongong CBD

Map 2 - Innovation Campus



Map 3 - Innovation Campus parking



Map 4 – Free Shuttle Bus