MONASH University

MONASH
MEDICINE
NURSING AND
HEALTH SCIENCES

2017 CCS 10th annual Graduate Research Symposium

Of the students, by the students, for the students

www.monash.edu/medicine/ccs/education/current-students/hdr-symposium

Cover image: Monash University Department of Diabetes laboratory work

Booklet compiled by Eliza Watson

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Introduction

2017 Postgraduate Symposium at The Alfred

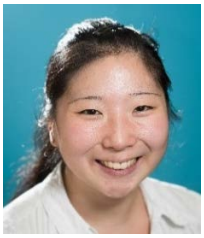
The main aim of the Central Clinical School's (CCS) symposium is to improve the visibility of students and their research projects on a larger scale and celebrate their achievements.

This symposium gives an opportunity for students to explain their research to other students and enables fostering of collaborations, networking and a greater awareness of the expertise and research being conducted on the site.

This is a student run event. The 2017 Postgraduate symposium planning committee members are:

Chair:

Mr Annas Al-Sharea



Vice-Chair:

Ms Minhee Halemba

Ms Michelle Flynn (Baker)

Mr Matthew Snelson (Diabetes)

Mr Muthu Mohan (Diabetes)

Ms Lakshanie Wickramasinghe (Immunology & Pathology)

Mr Paul Gill (Gastroenterology)

Student oral and poster presentations will be judged by a panel of senior academics and postdocs, with monetary prizes given for outstanding work.

Prizes for outstanding work

- Most outstanding oral presentation: **$400**
- Most outstanding poster presentation: **$400**
- Second place oral presentation: **$200**
- Second place poster presentation: **$200**
- Third prize oral presentation **$100**
- Third prize poster presentation **$100**
- Door prize* **$50**
- Student raffle prize** **$50**

*There are 3 sessions and one ticket is given per person per session (2 x oral presentation and 1 x poster presentation).

**Raffle tickets will be issued to students who ask questions (1 ticket per question). So, the more questions, the higher chance of winning!

2016 Postgraduate Symposium Committee: L-R, Ms Riya Palchaudhuri (Burnet Institute), Ms Erica Kim (Medicine), Mr Speros Thomas (Medicine), Ms Ashlee Conway (chair), Mr Annas Al-Sharea (Baker Institute), Ms Kirsty Wilson (Vice-chair), Ms Sin-Ki Ng (MAPrc).

Program

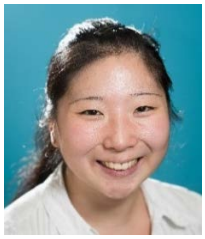
Friday 17th November 2017	
10.00-10.10 am	**Introduction & Welcome: Mr Annas Al-Sharea, Chairman student committee**
Session 1: Oral presentation Chair: Ms Minhee Halemba	
10.10-10.20 am	Speaker: Miss Ashlee Conway
10.20-10.30 am	Speaker: Miss Pia Varsamis
10.30-10.40 am	Speaker: Mr Mitchell Moon
10.40-10.50 pm	**Morning coffee break**
10.50-11.00 am	Speaker: Miss Jessica Marshall
11.00-11.10 am	Speaker: Ms Runa Lindblom
11.10-11.20 am	Speaker: Ms Riya Palchaudhuri
11.20-11.30 am	Speaker: Mr Daniel So
Session 2: Explain my graph Competition Chair: Mr Matthew Snelson	
11.30-12.00 pm	**Participants**: • Dr Graeme Lancaster (Baker) • Prof Merlin Thomas (Diabetes) • A/Prof Mark Wright (Immunology) • A/Prof Matt McCormack (ACBD) **Judges:** Committee
12.00-1.10 pm	**Lunch accompanying poster display (as listed under Session 3 below)**
Session 3: Poster presentations Chair: Ms Lakshanie Wickramasinghe and Mr Paul Gill	
(01)	Presenter: Miss Ee Fang Yu
(02)	Presenter: Mr Matthew Snelson
(03)	Presenter: Mr Shayden Bryce
(04)	Presenter: Miss Amy Searle
(05)	Presenter: Ms Erica Kim
(06)	Presenter: Miss Michelle Flynn
(07)	Presenter: Ms Minhee Halemba
Session 4: Oral presentation Chairs: Mr Annas Al-Sharea	
1.10-1.20 pm	Speaker: Mr Pacific Huynh
1.20-1.30 pm	Speaker: Ms Maria Selvadurai
1.30-1.40 pm	Speaker: Ms Rebecca Loh
1.40-1.50 pm	Speaker: Ms Mary Ajamian

1.50-2.00 pm	Speaker: Mr Paul Gill
2.00-2.10 pm	Speaker: Ms Elizabeth Thomas
2.10-2.20 pm	Speaker: Ms Christina Heris
Session 5:	**"No-Bell Prize" Competition for Supervisors**
2.20-2.40 pm	**Participants**: ● Prof Karin Jandeleit-Dahm (Diabetes) ● A/Prof Judy de Haan (Baker) ● Prof Andrew Perkins (ACBD) **Interviewing panel**: Mr Muthu Mohan and Ms Michelle Flynn **Judge/Timekeeper**: Mr Annas Al-Sharea
2.40-2.50 pm	Representative from MPA
3.00-3.20 pm	**Closing Remarks and Awarding of Prizes by Head of School, Central Clinical School, Prof Steve Jane**
3.30 pm	**Networking Drinks sponsored by MPA**

Judges for presentations

Judges for Oral presentations

Chair:
Mr Annas Al-Sharea



Chair:
Ms Minhee Halemba

Dr Christos Tikellis

Prof Rob Medcalf

Dr Jane Muir

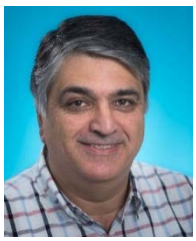
Dr Man Lee

Judges for Poster presentations

Chair:
Ms Lakshanie Wickramasinghe

Chair:
Mr Paul Gill

Dr Dan Andrews



Dr Philip Kantharidis

Dr Anna Watson

Dr Andrew Carey

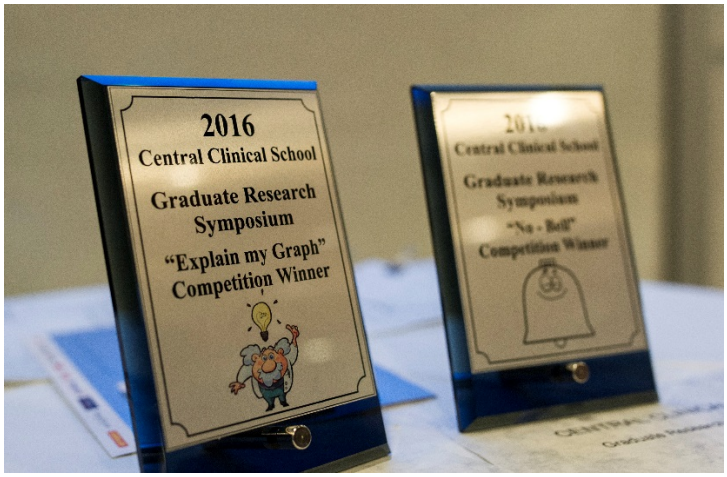
Supervisor sessions

No-Bell prize

Watch supervisors explain their complicated research without using any technical language! See how long they can go without ringing the bell. The interviewee who uses the least number of jargon words wins the session and a prize.

Explain my graph

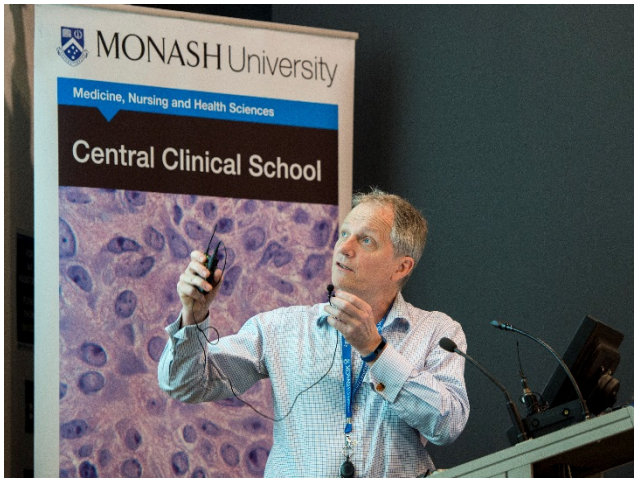
Supervisors are given 3-4 data and summary slides from outside their specialty area and have to present the slides to the audience. Each speaker has 5 minutes to present the slides followed by 2 minutes of questions from the audience. The best presenter wins the session and a prize.



2016 Supervisor session winners

No-Bell prize: Dr Ricardo Ataide

Explain my graph: Professor David Tarlinton (pictured, right)



Oral Presentation abstracts

Miss Ashlee Conway

Australian Centre for Blood Diseases

Oh, the Iron-y!

Iron deficiency accounts for 50% of all clinical reports of microcytic anaemia. Majority of these cases are simply due to insufficient dietary iron and are treatable with supplements, but there are a number of patients with suspected iron deficiency who prove unresponsive to therapy and display blood indices paradoxical to their condition. These unresolved cases of anaemia prompted the idea that unknown congenital mutations can occur within the iron metabolism pathway—that diet is not the only problem—but the means to identify them remains insufficient with current clinical approaches.

Studies of mutagenised mice with inheritable microcytic anaemia has identified a key protein within the iron metabolism pathway that holds pathological potential, called the transferrin receptor (TfR). When the TfR is mutated or aberrantly expressed on erythroid progenitors, the resulting animal phenotype mirrors that rare subset of anaemic patients, presenting as microcytic anaemia with unusual serum results that conflict with the longstanding clinical definition of iron deficiency anaemia.

With novel tools like AMNIS flow-imaging cytometry, we can visually depict how TfR mutations directly result in red blood cell pathology in these mutant mice, and why iron therapy fails to amend their condition. Slight alterations to typical blood and serum tests have also identified the crucial missing markers which could improve the clinical diagnosis of patients with congenital iron metabolic defects.

Miss Pia Varsamis

Baker Institute

Differing compositions and potential health impacts of sugars in popular soft drinks from Australia, Europe and the U.S.A.

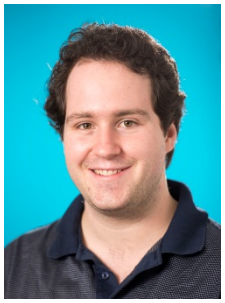
Purpose: Soft drink sweetening agents around the world (fructose, glucose and sucrose), activate distinct metabolic pathways that impact on glycaemic control. We compared the composition of sugars in four popular soft drinks currently sold in Australia, Europe and the U.S.A.

Methods: Sixty soft drink samples, marketed under the same trade name (Fanta, Sprite, Coca-Cola and Pepsi) in Australia, Europe and the U.S.A. (5 samples per drink per region) were analysed by an independent, certified laboratory (National Measurement Institute, Australia) for the concentration of sugars, using high-performance liquid chromatography. Comparisons for each drink type between regions were made using one-way ANOVA and where significant, individual means were compared with a least significant post-hoc test.

Results: Australian soft drinks were on average 22% higher in total glucose concentration (0.96 [SD = 0.22] g/100mL) than the U.S.A. formulations (4.44 [0.09] g/100mL; $p<0.001$). Most of the glucose in Australian formulations was contained within sucrose. European soft drinks were generally similar in

total glucose concentration to Australian formulations, and were 23% (1.04 [0.34] g/100mL) higher than U.S.A. formulations ($p<0.001$). Compared to the U.S.A. (6.39 [0.08] g/100mL), fructose concentration was lower in Australia (by 0.97 [0.28] g/100mL; $p<0.01$) and Europe (by 0.89 [0.35] g/100mL; $p<0.01$), respectively.

Conclusions: Australian soft drink formulations contain higher concentrations of total glucose, whereas those in the U.S.A. have greater amounts of total fructose. Since glucose, but not fructose, elevates blood glucose and insulin, Australian formulations may have a distinct long-term impact on glycaemic control for habitual soft drink consumers.



Mr Mitchell Moon

Australian Centre for Blood Diseases

A new approach to improve the safety profile of anti-thrombotic therapy: inhibition of PI3KC2α prevents thrombosis without impairing canonical platelet activation

Arterial thrombosis causes heart attacks and most strokes and is the leading cause of death worldwide. Platelets are the cells that form arterial thrombi, and anti-platelet drugs are the mainstay of heart attack and stroke prevention. Current drugs have limited efficacy, as well as clinically significant effects on bleeding. The key limitation on the ability of current drugs to impair thrombosis without causing bleeding is that they block global platelet activation, thereby indiscriminately preventing platelet function in haemostasis and thrombosis.

Here, we identify an approach that overcomes this limitation by preventing platelet function independently of canonical platelet activation, specifically in the setting of thrombosis. We have previously shown that genetic deficiency of the Class II PI3-kinase, PI3KC2α, alters the structure and function of the platelet internal membrane reserve and is anti-thrombotic (Mountford et al, Nat Comm, 2015). Here, we develop first-generation PI3KC2α inhibitors and show that pharmacological targeting of PI3KC2α in human platelets reproduces the effects of genetic deficiency in mouse platelets. Furthermore, PI3KC2α inhibitors are potently anti-thrombotic in human blood ex vivo and mice in vivo, without impairing activation-dependent platelet function or impacting haemostasis. Mechanistic studies reveal this anti-thrombotic effect occurs via a unique mechanism involving the regulation of membrane-dependent platelet adhesive function in the presence of hemodynamic forces.

These findings demonstrate an important role for PI3KC2α in regulating platelet structure and function via a unique membrane-dependent mechanism, and suggest that targeting the platelet internal membrane may be a suitable approach for novel anti-thrombotic therapies that exhibit limited bleeding.

Miss Jessica Marshall

Baker Institute

Characterisation of an Alzheimer's Disease mouse model expressing amyloid in the

presence of Tau: an extended replication study

Alzheimer's disease (AD) in Australia is the second leading cause of death; affecting over 300,000 patients with 1.2 million people involved in their care. Current research into therapies and treatments have been hampered by the availability of an appropriate and physiologically accurate mouse model of AD.

We have performed a comprehensive battery of behavioural and physiological tests on an AD mouse model developed by Heraud et.al in 2014. We crossed 5xFAD (overexpressing human APP and Presenilin-1) and Tg30 (overexpressing tau) mice so that we could investigate the effects of tau accumulation in the presence of amyloid pathology, which closely resembles human AD. In agreement with Heraud and colleagues we observed a decline in Rotarod performance at 7 months of age compared to wildtype. This corresponded with a 40% decrease in survival rates by 10 months. While we noted a decrease in Morris Water Maze performance at 8 months, Y-maze, large open field and novel object recognition tests were not yet significantly affected at this time point. Additionally, body weight was lower in the 5xFAD*Tg30 animals, with a significant difference in tibialis anterior skeletal muscle weight and tibia length. Our results successfully reproduced many aspects of the 2014 Heraud paper, while adding further additional characterisation of the model.

This initial characterisation study suggests that the model contains many aspects associated with frailty as well as the potential initiation of cognitive decline. Further characterisation is warranted to study the cognitive function in these mice with alternative tests and time points.

Ms Runa Lindblom

Department of Diabetes

The role of mitchondria in diabetic kidney disease

Diabetes is one the most important global health issues of the 21st century. Approximately 30% of patients will develop diabetic kidney disease (DKD). Few effective therapies are available to slow the progression of DKD to End Stage Renal Disease. Much of the underlying aetiology of DKD remains unknown, however mitochondrial dysfunction is co-associated with the disease. Studies from our laboratory have shown a decline in mitochondrial function in the kidney in DKD, leading to ATP depletion, changes in mitochondrial morphology and renal fibrosis. In addition, a greater susceptibility to mitochondrial permeability transition (mPT) pore opening is observed. In this study, we determined whether reducing mPT pore capacity would ameliorate diabetes-induced renal injury using both gene knockout and pharmacological strategies to directly interrupt mPT pore function.

In study 1, we used mice with a genetic knockout of the functional unit of the pore, Cyclophilin D (Ppif -/-). Wild type and Ppif -/- mice (n=15/group) were rendered diabetic using streptozotocin (55mg/kg/day, a model of T1D) and followed for 20 weeks. In study 2, the T2D db/db mouse and their littermate controls (db/h) were randomized to receive a Cyclophilin D inhibitor, the non-

immunosuppressive Cyclosporin A analogue, Debio-025, by daily oral gavage (10mg/kg/day) for 16 weeks.

Preliminary data show that neither the genetic deletion of cyclophilin D, nor its therapeutic targeting using Debio-025 reduced renal injury, with no change in albuminuria or glomerular sclerosis observed. This study suggests that the mPT pore is not a suitable therapeutic target in DKD.

Ms Riya Palchaudhuri

Department of Medicine

Towards a point-of-care for sepsis-biomarkers of inflammation

Introduction: Improved biomarkers are required for the detection of sepsis, both in laboratory settings and at the point of care (POC). Upregulation of surface CD64 expression (the neutrophil CD64 index, nCD64i) has been extensively studied as a sepsis biomarker with around 80% sensitivity, but is not amenable to use at POC. We hypothesised that simultaneous measurement of neutrophil CD64 and a neutrophil-specific protein in whole blood could yield a surrogate of the nCD64i that might be feasible for development of a POC test. To test this hypothesis, we evaluated the relative levels of total CD64 and neutrophil elastase in whole blood of healthy controls and patients with clinically diagnosed sepsis.

Methods: We are recruiting adult ICU patients (n=50) with clinically suspected sepsis (Alfred ICU) and healthy individuals (n=50). Levels of a selected neutrophil specific marker, neutrophil elastase (NE), and neutrophil activation marker (CD64) are measured by commercial sandwich ELISA kits. Samples were also tested using the Leuko64TM assay (Trillium Diagnostics) (surface CD64), and total (surface and intracellular) expression of CD64 was confirmed by flow cytometry staining, and microscopy.

Results: A strong correlation between total (whole blood) CD64 and NE was observed in healthy individuals (n=30, $R2=0.7$, $p<0.0001$), even without depletion of monocytes. We established assay cutoffs (mean+ 2SD) for CD64 and NE as well as a “Gating” strategy for healthy levels of CD64/NE for the samples with very low neutrophil count. The commercial Leuko64 kit (surface staining by flow) was positive in 20/25 patients. Conversely, total CD64 was highly elevated in most sepsis patients (23/25 positive, $p<0.0001$ compared to healthy controls), and was elevated compared to the healthy CD64/NE ratio in the remaining two patients (total 25/25 positive, $p=0.052$ compared to Leuko64). Flow cytometry using intracellular staining showed that a significant proportion of neutrophil CD64 was intracellular in patients negative by Leuko64.

Conclusion: We observed high correlation of CD64 and NE in controls, compared with the presence of elevated amounts of total CD64 (including intracellular CD64) and/or NE in whole blood of sepsis patients. Our results suggest that measurement of both total CD64 and NE levels in whole blood has promise as an improved candidate biomarker for diagnosis of sepsis

Mr Daniel So

Oral Presentation Abstracts

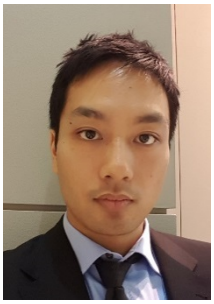
Department of Gastroenterology

Sugarcane fibre – understanding of efficacy and value creation

Disturbances to the composition of the gastrointestinal microbiota have been linked with a number of chronic diseases, presenting a potential modifiable risk factor the development of these conditions. Dietary fibre can potentially modulate the composition of the gastrointestinal microbiota by selectively stimulating beneficial bacteria such as Bifidobacterium and Lactobacillus species. This systematic review and meta-analysis aimed to assess the effect of fibre on gastrointestinal microbiota composition.

A structured search of MEDLINE, EMBASE, CINAHL and CENTRAL was conducted (up to October 2017) for randomised controlled trials evaluating the effect of dietary fibre intervention on the gastrointestinal microbiota in healthy adults. Meta-analyses were performed using random-effects model on pre-specified bacterial abundances including Bifidobacterium and Lactobacillus spp., comparing dietary fibre interventions with placebo and low fibre comparators. A total of 64 studies encompassing 2,099 participants were included.

Dietary fibre intervention led to higher faecal abundances of Bifidobacterium [Standardised Mean Difference (SMD) 0.64 (95% CI: 0.42, 0.86), P < 0.001] and Lactobacillus spp. [SMD 0.22 (95% CI: 0.03, 0.41), P = 0.02] compared with placebo and low fibre comparators. Subgroup analysis revealed only dietary fibre interventions involving fibres defined as prebiotic led to significantly higher abundances of Bifidobacterium (P < 0.001) and Lactobacillus spp. (P = 0.002) compared with placebo and low fibre comparators, while interventions using general fibres (e.g. wheat bran) had no effect. Increased intake of prebiotic fibre selectively manipulates gastrointestinal microbiota composition, which may benefit host health by decreasing risk of developing chronic disease, although confirmation from longitudinal studies is required.



Mr Pacific Huynh

Department of Diabetes

CDA1 deficiency exacerbates renal Ischaemia/Reperfusion (IR)-induced proinflammatory gene expression

The development of fibrosis, the common final pathological feature of most chronic kidney diseases, is partially mediated by the actions of Cell Division Autoantigen 1 (CDA1). In fact, global genetic deletion of CDA1 has previously been demonstrated to attenuate renal fibrosis in a mouse model of diabetic nephropathy. The pathological role of CDA1 in the development of non-diabetic renal fibrosis has yet to be examined experimentally. Thus, to investigate the antifibrotic potential of targeting CDA1 in non-diabetic kidney disease, this study examined the effect of the genetic deletion of CDA1 in a mouse model of acute renal injury, the renal ischaemic/reperfusion (IR) injury.

Male CDA1 WT and KO had their left renal artery was ligated for 45 mins and then released. Mice were then followed for 4 and 8 days' post-obstruction, before kidneys were extracted for histological and biochemical analysis.

IR injury was associated with an upregulation in many profibrotic and proinflammatory genes in CDA1 mice, including increases in expression of Col III, fibronectin and MCP1 by

~35.3, ~16.2 and ~37.2 fold respectively ($p<0.01$ vs Sham CDA1 WT 4 IR mice). Despite expectations, CDA1 KO mice were not associated with any reductions in profibrotic gene expression in this model. Interestingly, however, CDA1 deficiency appears to exacerbate proinflammatory gene expression, with increases in gene expression of TNFα, iNOS, and IL6 by ~241%, ~399%, and 753%, respectively, at four days post-IR injury compared to CDA1 WT mice ($p<0.05$). Thus, CDA1 deficiency exacerbates proinflammatory gene expression in the renal IR injury model.

Ms Maria Selvadurai

Australian Centre for Blood Diseases

Targeting the platelet membrane reveals a novel approach for improved anti-platelet therapies

Background: The class II PI3K, PI3KC2a, is a broadly expressed lipid kinase with emerging biological roles. We have recently shown that PI3KC2a is important in platelet structure and function in mouse - PI3KC2a-deficient mice have impaired thrombotic function that appears due to a dysregulated open canalicular system (OCS) structure. We have subsequently developed a novel pharmacological inhibitor of PI3KC2a, X151, allowing us to examine whether a similar role for PI3KC2a exists in human platelets.

Aim: To determine the role of PI3KC2a in the regulation of human platelet membrane structure and function, and the viability of targeting PI3KC2a as an anti-thrombotic strategy.

Methods: The structural impact of PI3KC2a deficiency and inhibition on the OCS was assessed using TEM and SEM in 2D, and FIB-SEM in 3D. Ex vivo thrombosis in human whole blood, and in vivo thrombosis in mouse models, were used to assess impact on prothrombotic function.

Results: PI3KC2a inhibition with X151 in human platelets caused enlargement of the OCS leading to an increased OCS volume, similar to the effect seen in PI3KC2a-deficient mouse platelets. Changes in the number and size of OCS openings at the plasma membrane were also observed. PI3KC2a inhibition significantly reduced thrombus formation in an ex vivo human whole blood model and two distinct in vivo mouse models.

Conclusion: These findings demonstrate that PI3KC2a is involved in the regulation of platelet membrane structure and function in both mouse and human, and suggest that targeting the platelet membrane via PI3KC2a may provide potential as a novel anti-thrombotic drug target.

Ms Rebecca Loh

Baker Research Institute

Pioglitazone reduces cold-induced brown fat glucose uptake despite induction of browning in cultured human adipocytes: a randomised, controlled trial in humans

Increasing energy expenditure via brown adipose tissue (BAT) thermogenesis is a possible strategy to manage obesity and associated co-morbidities. Thiazolidinediones (TZD) increase BAT differentiation and

recruitment in pre-clinical experimental models, but whether these actions extend to humans in vivo is unknown. The aim of this study was to determine the effect of pioglitazone treatment on adipocyte browning and adaptive thermogenesis in humans.

We first demonstrated that pioglitazone treatment of cultured human primary subacromiclavicular-derived adipocytes induced browning. The effect of pioglitazone (45mg daily, 28 days) on BAT activity was then assessed in a double-blind, placebo-controlled, parallel trial, involving 14 lean, healthy males. Pioglitazone reduced cold-stimulated BAT activity relative to placebo and increased total and lean body mass. There was no effect of pioglitazone on energy expenditure, cardiovascular responses, core temperature, blood metabolites or hormones under either basal conditions or in response to acute cold exposure. The disparate actions of pioglitazone on BAT between preclinical experimental models and our in vivo human trial highlight the imperative to conduct human proof-of-concept studies as early as possible in BAT research programs aimed at therapeutic development.

Our clinical trial findings suggest reduced BAT activity may contribute to weight gain associated with pioglitazone and other TZDs.

Ms Mary Ajamian

Department of Gastroenterology

The Utility of Serum Zonulin, the "Leaky Gut" Protein, as a Marker of Gastrointestinal Dysfunction

Background: Dysregulation of the zonulin pathway has been linked to a “leaky gut” due to the protein zonulin’s putative role as a modulator of intestinal epithelial tight junctions. Zonulin is a proposed contributor of non-coeliac gluten sensitivity/IBS (NCGS), coeliac disease (CD) and inflammatory bowel disease (IBD) pathogenesis. However, controversy lies in zonulin’s utility as a serum biomarker of disease and in the assays used for its assessment.

Aims: To characterize serum zonulin levels amongst patients with gastrointestinal conditions and healthy individuals and verify commercially-available assays for zonulin quantification.

Methods: Serum zonulin was measured in patients with NCGS (n=36), CD (n=37), and IBD (n=20), as well as healthy subjects (n=49) by CUSABIO ELISA. Zonulin non-producers were determined by western blot-based haptoglobin phenotyping. Zonulin levels in selected sera were compared with those determined by another commercially-available assay (Immundiagnostik) and both assays were spiked with recombinant zonulin for verification.

Results: 32 of 36 patients with NCGS, 34 of 37 with untreated CD, 19 of 20 with IBD, and 46 of 49 healthy subjects were zonulin producers. Compared with healthy subjects (median 0.00 ng/mL, IQR 0.00 ng/mL), patients with NCGS (0.32, 0.90), CD (0.07, 1.27), and IBD (1.73, 2.17; all $p<0.0001$) had elevated serum zonulin. Levels in IBD were higher than those in NCGS ($p=0.004$) and CD ($p=0.005$) with no differences between NCGS and CD. However, non-producers had protein levels detected by CUSABIO assay, recombinant zonulin was not detected by either assay, and there was poor correlation between the results of both assays ($n=28$; $r= 0.29$; $p=0.14$).

Conclusion: Serum zonulin levels measured through CUSABIO assay are elevated in patients with gastrointestinal conditions, though there was poor verification for both commercial assays. Rather than support a role

for zonulin in intestinal disease pathogenesis, the current results cast doubt upon the validity of commercially-available zonulin assays.

Mr Paul Gill

Department of Gastroenterology

Quantification of short chain fatty acids in fermented food and beverages

Short chain fatty acids (SCFAs) are produced within the colon as the result of colonic fermentation of 'dietary fibre'. SCFA promote gut health and also have systemic effects. Increased delivery of SCFA to the body may be desirable. SCFAs are also present in many fermented foods and beverages, representing a poorly characterised oral source of SCFA. We aimed to quantify the SCFA levels present within fermented products to determine their potential for therapeutic use.

Commonly consumed and readily available fermented foods and beverages were chosen for analysis. Representative samples of food and beverages were collected and pooled. SCFA were quantified using gas chromatography with a flame ionization detector (GC-FID). Heptanoic acid was used as an internal standard. Dairy products were extracted using diethyl ether with a valeric acid internal standard. Samples were run in triplicate with variation <5%. Sample data was expressed as mg/g as well as mg per serving size. Acetate was the most abundant SCFA detected and was highest in pepper sauce (124 mg/g) and apple cider vinegar (67 mg/g). Blue vein cheese (5.5 mg/g) and Gherkin (0.6 mg/g) contained the highest levels of butyrate and propionate respectively. After adjusting for serving sizes, kombucha (1200 mg) and apple cider vinegar (1000 mg) provide the most SCFA, mainly as acetate.

Oral sources of SCFA via the consumption of fermented foods may provide another approach for elevating circulating levels of SCFA. The potential immune-modulating and epigenetic effects require further investigation.

Ms Elizabeth Thomas

Monash Alfred Psychiatry Research Centre

Memory guided saccade performance across the schizophrenia continuum

Saccadic (ocular motor) deficits are one of the most replicated findings in schizophrenia. However, less research has been conducted investigating the broader schizophrenia continuum. Recent research suggests that the personality characteristics and symptoms observed in schizophrenia lie on a continuum with subclinical symptoms, known as schizotypy, observed in the non-clinical population. As saccadic deficits are a cognitive hallmark of schizophrenia, it is believed that saccadic deficit may be associated with higher schizotypy. This study investigated saccadic performance across the schizophrenia continuum using the memory-guided (MG) saccade paradigm as no studies to date have investigated MG performance in schizotypy.

43 patients with schizophrenia/schizoaffective disorder and 93 healthy controls completed the MG saccade task, which engages spatial working memory and inhibition processes. Schizotypy was assessed using the Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE) questionnaire, which

measures the four schizotypy factors: unusual experiences (UnEx), introvertive anhedonia (InAn), cognitive disorganisation (CogDis) and impulsive nonconformity (ImpNon). MG latency and error rate were significantly different between patients and controls ($p<0.001$). Looking across the schizophrenia continuum, there were significant correlations between MG latency and UnEx ($p<0.001$), InAn ($p=0.047$) and CogDis ($p=0.001$), as well as with the O-LIFE total score ($p<0.001$). There was also a non-significant trend between MG error and O-LIFE total score ($p=0.086$), though no significant correlations were observed with any schizotypy factor scores.

This is the first study to investigate and demonstrate the relationship between higher schizotypy and impaired MG performance. The findings also support the theory of schizotypy and a broader schizophrenia continuum.

Ms Christina Heris

Department of Epidemiology and Preventive Medicine

Living life smoke-free: the social and environmental context of urban Aboriginal adolescents in NSW

Objectives: To describe the social, cultural and environmental factors associated with smoking and non-smoking behaviour among Aboriginal adolescents.

Methods: Cross-sectional analysis of baseline responses from 120 Aboriginal adolescents (12-17y) and their carers from urban NSW in the Study of Environment on Aboriginal Resilience and Child Health (SEARCH cohort study). Outcomes of interest include ever smoked and never smoked status. Logistic regression was used to measure associations between smoking status and demographics, substance use, social and emotional wellbeing, exposure to environmental tobacco smoke, community and cultural connection, academic engagement and negative life experiences were examined.

Results: The majority (79%) have never smoked regularly with 18% ever having smoked regularly (mean age of commencement 12). Exposure to smoking was high with 72% report at least one smoking parent, 40% live with someone who smokes inside, 60% have a parent who smoked during pregnancy. Substance use, sexual activity, high SDQ scores, interactions with justice, housing instability, expulsion from school were all associated with increased odds of ever smoking. Having their mother as main carer, strong school engagement and community connections were significantly associated with having never smoked regularly.

Conclusions: Most Aboriginal adolescents have never smoked regularly despite high exposure to environmental tobacco smoke. There are several common life-stage risk factors. Protective factors cross individual, social and environmental levels of influence. Understanding protective factors associated with non-smoking status will be important for developing context relevant interventions to prevent early onset of smoking.

Poster Presentation abstracts

(1) Miss Ee Fang Yu

Baker Institute

Molecular Targeting of Inflammation for Diagnosis and Therapy

Inflammation contributes to development of many chronic diseases such as autoimmune and cardiovascular diseases. Mac-1 is expressed on polymorphonuclear leukocytes such as monocytes and contributes to inflammation. DARPinF7 (Designed Ankyrin Repeat Protein F7) is a novel binding protein that specially binds to the ligand binding domain, I domain of Mac-1, that is exposed upon activation.

AIM: To develop targeted diagnostic and therapeutic agent for inflammation using DARPinF7.

METHODS: Three phage panning rounds of DARPin phage libraries against purified mouse I domain were performed to select targeted DARPin specific for I domain of Mac-1. Enzyme-linked immunosorbent assay (ELISA) was performed to determine binding of DARPinF7 to both purified mouse and human I domain. To determine binding of DARPinF7 to activated monocytes, flow cytometry was performed on mouse immortalised RAW264.7 macrophages, mouse fresh monocytes, human immortalised THP-1 macrophages, and human fresh monocytes that were activated by Phorbol 12-Myristate 13-Acetate (PMA).

RESULTS: DARPinF7 was selected based on ELISA on its binding to mouse I domain. ELISA showed significantly higher binding of DARPinF7 to purified mouse I domain ($p<0.0001$) and human I domain ($p<0.0001$) than DARPinE3_5. Flow cytometry also demonstrated greater binding of DARPinF7 than DARPinE3_5 to activated mouse RAW264.7 cells, mouse fresh monocytes, human THP-1 cells, and human fresh monocytes, as shown by higher fluorescence detection.

CONCLUSION: DARPinF7 specifically binds to activated Mac-1 on both mouse and human monocytes. It shows promising results for a novel targeting protein as a diagnostic and therapeutic agent to combat inflammatory diseases such as atherosclerosis and arthritis.

(2) Mr Matthew Snelson

Department of Diabetes

Resistant starch ameliorates advanced glycation endproduct-induced albuminuria in a mouse model of type 2 diabetes

Background/Aims: Heat treating foods leads to the formation of advanced glycation endproducts (AGEs) which contribute to chronic renal injury. Recent research implicates gut dysbiosis in the progression of diabetic nephropathy. This study investigates whether excess consumption of dietary AGEs causes gut dysbiosis, exacerbating renal injury in a type 2 diabetes mouse model.

Methods: Six week old diabetic (db/db) and non-diabetic (db/h) mice were randomised (n=12/group) to receive a low AGE (LAGE, unbaked rodent chow) or a high AGE diet (HAGE, baked at 160°C for 1 hour), with or without resistant starch (RS) for 10 weeks. 24-hour urine was collected and albuminuria was

measured. Intestinal permeability was assessed in vivo by the clearance of FITC-labelled dextran (500mg/kg body weight). Statistical differences were assessed by one-way ANOVA.

Results: The high AGE diet exacerbated albuminuria in db/db mice (874.4±154.8 vs 536.2±96.5µg/24h, P<0.05, db/db HAGE vs db/db LAGE), and RS attenuated this AGE-induced increase (874.4±154.8 vs 515.5±71.9µg/24h, P<0.05, db/db HAGE vs db/db HAGE+RS). Db/db mice had greater gut permeability compared to db/h mice (2.38±0.32 vs 1.05±0.11µg/ml, P<0.01, db/db LAGE vs db/h LAGE). Db/db-HAGE-fed mice trended towards increased gut permeability (3.43±0.43 vs 2.38±0.32µg/ml, P=0.06, db/db HAGE vs db/db LAGE), an effect not observed in RS-fed db/db mice.

Conclusions: Heat-treated diets led to increased intestinal permeability and worsening albuminuria in db/db mice. RS was protective against high AGE-induced albuminuria in db/db mice. These preliminary studies support the notion that dietary AGEs contribute to renal disease via alterations in gut homeostasis.

(3) Mr Shayden Bryce

Monash Alfred Psychiatry research centre

Cognitive remediation in schizophrenia: Impact on cognitive and psychosocial outcome and role of intrinsic motivation

Background: Cognitive remediation (CR) therapies represent a promising method of reducing cognitive deficits in schizophrenia. Nevertheless, there have been few controlled trials, and the extent to which CR generalizes to everyday self-efficacy and everyday functioning remains unclear. In addition, the role of patient-specific factors such as intrinsic motivation in predicting attendance and training outcomes have not been thoroughly investigated. This study aimed to address these limitations in an assessor-blinded, randomized and controlled trial comparing group-based 'drill-and-strategy' CR with a computer game (CG) control. Experimental design: Fifty-six people with schizophrenia were randomized into CR or CG, and offered 20 one-hour sessions over 10 weeks. Measures of cognition, psychopathology, self-efficacy, independent living skills were measured at baseline, end-group and three-months post-intervention. Intrinsic motivation was measured at baseline and end-group.

Results: CR completers demonstrated greater improvements in cognitive function than controls, with three-quarters (77%) of CR completers showing a reliable improvement in at least one cognitive domain. Both groups showed increased self-efficacy. No changes in independent living skills were observed. Early reports of program value predicted session attendance, while enhanced program interest and value over time increased the likelihood of reliable cognitive improvement. Implications: CR may improve cognition in schizophrenia when compared to active controls. Promoting greater motivation over time may increase the likelihood of achieving meaningful cognitive improvements. CR may not translate to independent living domains, however, even if cognition and everyday confidence are enhanced. Consequently, functioning may need to be targeted directly to achieve meaningful changes in this domain.

(4) Miss Amy Searle

Baker Institute

Targeted thrombolysis: novel, innovative, risk-free and site specific delivery of an anti-thrombotic therapeutic

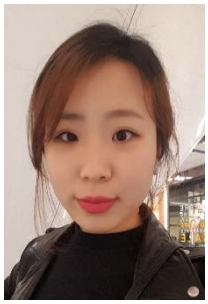
Myocardial infarction and ischaemic stroke remain the leading cause of mortality both in Australia and worldwide. Whilst therapeutic advances have been made to address these statistics, widespread use of these pharmaceutic therapies within the clinics has been hampered due to associated bleeding risk and complications.

Aim: To develop a targeted anti-thrombotic therapy to deliver a therapeutic payload directly to the site of the thrombus.

Methods: A recombinant fusion protein was designed, Targ-TAP, containing a single-chain antibody targeted towards the activated platelet GPIIb/IIIa integrin, scFvTarg, as well as the potent therapeutic inhibitor of Factor Xa, tick anticoagulant peptide (TAP). Using in vitro assays, as well as an in vivo model of thrombosis, the specificity and functionality of Targ-TAP as a novel thrombolytic therapeutic was assessed.

Results: Flow cytometry assays using both human and mouse blood demonstrated the specific binding of SCE5-TAP to ADP activated platelets. The specificity of Targ-TAP toward the GPIIb/IIIa integrin was demonstrated via a competitive assay, whereby the addition of Targ-TAP significantly inhibited anti-fibrinogen antibody binding. Flow chamber assays using bright field microscopy demonstrated the potent thrombolytic capacity of Targ-TAP as well as its specificity toward aggregated platelets. Further, using a FeCl3-induced mouse model of arterial thrombosis, Targ-TAP significantly delayed arterial occlusion and importantly, tail-bleeding assays determined SCE5-TAP had no effect upon bleeding time or blood volume loss.

Conclusion: Targ-TAP allows for a high concentration of the thrombolytic drug to be delivered directly to the thrombus, whilst ensuring low systemic concentrations. Targ-TAP demonstrates a novel therapeutic toward a safe, bleeding complication free treatment of thrombosis related diseases.



(5) Ms Erica Kim

Department of Neuroscience

Overcoming monocarboxylate transporter 8 (MCT8)-deficiency to promote human oligodendrocyte differentiation and myelination

Cell membrane thyroid hormone (TH) transport is primarily mediated by the monocarboxylate transporter 8 (MCT8). Human mutations of the gene, slc16a2, result in the X-linked-inherited psychomotor retardation and hypomyelination disorder, Allan-Herndon-Dudley syndrome (AHDS). We posited that abrogating MCT8-dependent TH transport limits oligodendrogenesis and myelination.

We show that human oligodendrocytes (OL), derived from the Nkx2.1-GFP human embryonic stem cell (hESC) reporter line, express MCT8. Moreover, treatment of these cultures with DITPA (an MCT8-independent TH analog), up-regulates transcription factors specific to OL differentiation and myelin gene expression. DITPA treatment promotes hESC-derived OL myelination of retinal ganglion

axons in co-culture. Pharmacological and genetic blockade of MCT8 induces significant OL apoptosis, impairing myelination. DITPA treatment reverses OL apoptosis mediated by slc16a2 down-regulation and promotes myelination.

Our results highlight the potential role of MCT8 in TH transport for human OL development and may implicate DITPA as a promising treatment for developmentally-regulated myelination in AHDS.

(6) Miss Michelle Flynn

Baker Institute

Transient intermittent hyperglycaemia promotes myelopoiesis and accelerates atherosclerosis in an S100A8/A9-RAGE dependent manner

Transient postprandial hyperglycaemia is an independent risk factor for cardiovascular disease. We have previously shown that chronic hyperglycaemia in diabetic mice increases myelopoiesis and atherosclerosis through S100A8/A9-dependent activation of via the receptor for advanced glycation end-products (RAGE).

Using a novel model mimicking transient intermittent hyperglycaemia (TIH) observed in adults with impaired glucose tolerance, we show here that TIH activates the same signalling pathways as chronic hyperglycemia to promote myelopoiesis in the bone marrow (BM), resulting in increased circulating monocytes, particularly the inflammatory Ly6-Chi subset, and neutrophils. Weekly recurrence of TIH also results in accelerated atherogenesis, comparable to lesion formation in diabetic mice with chronic hyperglycaemia. Haematopoietic deletion of S100a9-/-, S100a8-/- or its cognate receptor RAGE, affords protection against TIH-induced myelopoiesis, monocytosis and neutrophilia. Increased glycolytic rate in neutrophils via GLUT-1-dependent glucose uptake appears to promote the release of S100A8/A9, and deletion of this transporter in the myeloid lineage protects from TIH-induced myelopoiesis. Similarly, inhibiting S100A8/A9 using ABR-215757 reduces myelopoiesis in mice subjected to TIH, resulting in decreased circulating monocytes and neutrophils and smaller atherosclerotic lesions, with lower lipid and macrophage content.

Together, these data suggest that TIH accelerates atherogenesis by stimulating S100A8/A9 signalling through RAGE to promote myelopoiesis and generate monocytosis and neutrophilia, and this axis represents a potential target for vasculoprotective therapy.

(7) Ms Minhee Halemba

Australian Centre for Blood Diseases

Exploring acute myeloid leukaemia (AML) bioenergetics and drug sensitivity by rendering AML cell lines impaired for either Glycolysis or Oxidative phosphorylation

Acute myeloid leukaemia (AML) is an aggressive haematological malignancy that results in the overproduction of immature myeloid cells. While chemotherapy is the standard treatment, majority of patients relapse or are refractory to treatment, highlighting the need to develop new therapeutic options. Cancer cells are known to

undergo severe metabolic reprogramming, thus targeting AML aberrant bioenergetics could be a more effective therapeutic approach. The two main processes for energy production are glycolysis and oxidative phosphorylation. AML cell lines made deficient in either one of these metabolic pathways can be used to characterise AML metabolic reliance and establish a screen for drugs targeting each metabolic dependency.

Two AML cell lines have been treated with 2DG (a glycolytic inhibitor) or oligomycin (an inhibitor of oxidative phosphorylation), to obtain drug resistant cells that are either glycolysis- or oxidative phosphorylation-deficient, respectively. These cells have been tested for their acquired drug resistance in survival assays where increased survival was observed when the cells were treated with 2DG (for 2DG-resistant cells) or oligomycin (for oligomycin-resistant cells) compared to parental cells. The metabolic phenotype has also been assessed in metabolic assays that have established the impaired glycolysis and oxidative phosphorylation profile for the 2DG-resistant and oligomycin-resistant cells, respectively. These cells can now be used in drug screening where the glycolytic deficient cells would be sensitive to drugs that target oxidative phosphorylation while the oxidative phosphorylation deficient cells would be sensitive to glycolysis targeting drugs. The discovery of such drugs targeting both metabolic pathways can then be tested further in combination studies.

This study offers a new way to treat AML by uncovering new drug combinations able to target malignant metabolism bioenergetics.

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