BE WELL.
NOT A WISH. A PROMISE.

For more than 150 years, a very special passion has driven the people of MSD. Our goal is to develop medicines, vaccines, consumer care and animal health innovations that will improve the lives of millions. Still, we know there is much more to be done. And we’re doing it, with a long-standing commitment to research and development. We’re just as committed to expanding access to healthcare and working with others who share our passion to create a healthier world. Together, we’ll meet that challenge. Promise.
Dear Colleagues,

Welcome to State of the Heart 2014!

We are delighted that for the first time the annual scientific meetings of the International Society of Cardiovascular Pharmacotherapy (ISCP), the Australian Atherosclerosis Society (AAS), the High Blood Pressure Research Council of Australia (HBPRCA), the Australian Vascular Biology Society (AVBS) are being held as one, in conjunction with the Cardiovascular Special Interest Group of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT CV-SIG).

This combined meeting will provide a truly unique opportunity for scientific exchange on current topics in cardiovascular pharmacotherapy and actively promote interaction between delegates and faculty members. The mix of clinical and scientific sessions will be second to none. The organising committees have worked together to prepare an exciting programme that will include a rich mix of plenary lecture, parallel sessions and poster sessions on cutting edge issues and research.

Not only do we have this unparalleled opportunity to bring all Societies together in one place, but also that we have such a stunning backdrop of Adelaide to have these discussions.

On behalf of the Local Organising Committee, we welcome you to the State of the Heart meeting 2014 in Adelaide.

Best regards

Prof. Stephen Nicholls
President
Local Organising Committee

Prof. George Dan
President
ISCP
State of the Heart 2015
20th Annual Scientific Meeting of the International Society of Cardiovascular Pharmacotheraphy (ISCP)
Argentine Catholic University - Buenos Aires, Argentina, 25-26 June 2015

COMMITTEES
Local Organizing Committee - Chairman: Alvaro Sosa Lpirandi - Alberto Lorencetti
Cardiovascular Clinical Trialists Forum - Chairman: Faez Zarrad
Cardiovascular Prevention Symposium - Chairman: Valentin Fuster

INTERNATIONAL COMMITTEE
George Dan (President ISCP)
Felipe Martinez (President Elect ISCP)
Juan Carlos Kaski (Chairman Executive Board ISCP)

MAIN TOPICS
Established and emerging lipid lowering drugs in face of new guidelines
Pharmacologic management of hypertension 2015
New drugs for heart failure
Evidence based use of anticoagulants in atrial fibrillation
Device vs drug management of arrhythmias
New therapeutic approaches in diabetes
Innovative therapeutic strategies: polypill
Vaccines as cardiovascular prevention strategy

mci margarita.perkins@mci-group.com +54 11 5252 3154

KEY DATES
ABSTRACTS SUBMISSION
Opens: 1 Dec 2014
Close: 31 March 2015

EARLY BIRD REGISTRATION
Opens: 1 Dec 2014
Close: 31 March 2015

WORLD HEART FEDERATION
Associate International Member
www.iscpcardio.org
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Executive Committees

Local Organising Committee State of the Heart 2014

Stephen Nicholls (President)
Philip Aylward
Alex Brown
Stephen Worthley
John Beltrame
Prash Sanders

ISCP Board of Directors/Executive Committee

George Dan (President)
Felipe Martinez (Vice President/President Elect)
Juan Carlos Kaski (Past President)
Dennis Cokkinos (Secretary)
Augusto Gallino (Treasurer)

AAS Executive Committee

Stephen Nicholls (President)
Karin Jandeleit-Dahm (Past President)
Trevor Mori (Secretary)
Christina Bursill (Treasurer)
Heather Medbury (Assistant Treasurer)
Andrew Murphy (Program Secretary)
Steven Gieseg (Membership Secretary)
Fatiha Tabet (Newsletter Editor)

AVBS Executive Committee

Karlheinz Peter (President)
Mary Kavurma (Secretary)
Elizabeth Gardiner (Treasurer)
Simone Schoenwaelder
Andrew Murphy
Marc Achen (2014 Program Chair)
Peter Little (Immediate Past President)

HBPRCA Executive Committee

Jaye Chin-Dusting (President)
Rob Widdop (Secretary)
Clive May (Treasurer)
Anne Barden (Program Secretary)
Janna Morrison (Membership Secretary)
James Sharman (Website Editor)
Brian Morris (Abstract Editor)
Geoff Head (Corporate Liaison)
Karin Moritz (Society Liaison)
Mark Nelson (Clinical Liaison)
Francine Marques (Young Investigator)
The International Society of Cardiovascular Pharmacotherapy (ISCP) is a Global organisation with a large membership from over 30 countries worldwide. They aim at promoting and facilitating strategies to improve cardiovascular health through promoting education and research in all areas of cardiovascular pharmacotherapy with the ultimate goal of preventing and improving treatment, clinical outcome and patient wellbeing.

The Australian Atherosclerosis Society (AAS), formed in 1974, promotes, at a national level, the advancement of science, research and teaching in the field of atherosclerosis. The AAS endeavours to achieve these objectives by promoting the exchange of existing knowledge; encouraging new research ventures and interdisciplinary approaches; and fostering the dissemination of knowledge by organising national and international scientific meetings. Membership is open to researchers in the field of atherosclerosis and cardiovascular disease.

The Australian Vascular Biology Society is an organization active throughout Australia and New Zealand which aims to foster research communication by scientists and clinicians from a broad range of disciplines but with a unifying interest in biology of the cardiovascular system. The main areas of interest include atherosclerosis, inflammation, thrombosis, angiogenesis, endothelial function, hypertension and diabetes. Members receive newsletters with current items of vascular biology interest, including notification of national and international vascular biology meetings, and are also eligible for Society-sponsored travel grants to these meetings.

The High Blood Pressure Research Council of Australia has been at the forefront of research into the causes, prevention and treatment of high blood pressure since its inception in 1979. Our research incorporates the full range from experimental molecular biology and genetics to human physiology and drug treatment of hypertension. Its members are among the national and international leaders in the field of cardiovascular research, through clinical trials, research projects, journal editorships, various academic, government and commercial committees and as active organisers and participants at major conferences.

The Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) is the professional and independent Society in Australia and New Zealand with expertise in the use and toxicity of medicines and chemicals. ASCEPT was founded in 1966, and held its first scientific meeting a year later. Since that time, ASCEPT has developed into the peak professional society devoted to advancing excellence in Clinical and Experimental Pharmacology and Toxicology in Australasia.
Congress Information

Registration Desk
Registration/enquiries are welcome at the registration desk. The congress team will be in attendance as follows:

Wednesday 26th November  08.30 – 18.00
Thursday 27th November  08.00 – 18.00
Friday 28th November  08.00 – 16.00

Important Dates/Lectures:

**Wednesday 26th November**
16.45 – 17.15  Colin I Johnston Lecture – Tom Marwick (Hall D)
17.45 – 19.00  Welcome Reception (Halls JK)

**Thursday 27th November**
12.30 – 13.15  AAS AGM (RB 3)
13.30 – 14.00  ISCP Invited Lecture – Harvey White (Hall D)
16.45 – 17.45  HBPRCA AGM (RB 3)
17.45 – 18.45  AVBS AGM (RB 1/2)
19:30 – 21.30  Delegate dinner at the SAHMRI

**Friday 28th November**
10.45 – 11.15  Austin Doyle Lecture – Brian Schmidt (ANU Nobel Laureate)
13.30 – 14.00  RD Wright Lecture – Rhian Touyz (Hall D)
14.00 – 14.30  Awards Ceremony (Hall D)

Abstract Reviewers

**ISCP**
George Dan, Romania
Felipe Martinez, Argentina
Juan Carlos Kaski, UK
Dennis Cokkinos, Greece
Augusto Gallino, Switzerland
Thomas Kahan, Sweden

**AAS**
Karin Jandeleit-Dahm (Baker IDI)
Stephen Nicholls (SAHMRI)
Trevor Mori (University of Western Australia)
Christina Bursill (Heart Research Institute)
Heather Medbury (University of Sydney)
Andrew Murphy (Baker IDI)
Fatiha Tabet (University of NSW)
Steven Gieseg (University of Canterbury)

**AVBS**
Grant Drummond (Monash University)
Mary Kavurma (Heart Research Institute)
Chris Sobey (Monash University)
Shane Thomas (University of NSW)
**Speaker Information**

**HBPRCA**  
Anne Barden (University of Western Australia)  
James Sharman (Menzies Institute for Medical Research)  
Duncan Campbell (St Vincent's Institute of Medical Research)  
Stephen Harrap (University of Melbourne)  
Geoff Head (Baker IDI)  
Mark Nelson (University of Tasmania)  
Clive May (Florey Institute of Neuroscience and Mental Health)  
Janna Morrison (University of South Australia)  
Michael Stowasser (University of Queensland)  
Brian Morris (University of Sydney)  
John Chalmers (The George Institute for International Health)  
Lindon Wing (Flinders University)  
Doug McKitrick (University of Western Australia)  
Marcus Schlaich (University of Western Australia)

**Presentations**

**Speakers**  
All speakers are requested to upload their presentations to the Speaker Service Centre located in Lounge C, at least 2 hours ahead of the time of presentation. AV technicians will be on hand to assist.  
All presenters should familiarise themselves with the audio-visual equipment in their allocated room well before their presentation. The AV technicians and congress team are available to provide assistance during any of the refreshment breaks.

**Posters**  
All posters will be displayed in Halls JK of the Adelaide Convention Centre. Posters are to be displayed from 9am on Wednesday 26th November and should remain in place until the conclusion of the meeting.

Presenting authors should be in attendance for 30 minutes during coffee breaks and from 12.45-13.15 during lunch breaks. Please check the final number of your poster in the abstract book.

**General Information**

**Mobile phones, pagers** - as courtesy to speakers and other delegates, please switch off these devices during sessions or switch them to vibration mode.

**Dress code** - Dress for conference sessions is informal (smart casual).

**Disclaimer** - The Organising Committee reserves the right to make program changes if deemed necessary.

**Name badges** - Conference delegates are requested to wear their name badges to all conference activities.

**No Liability** - In the event of any disruption or event leading to losses or added expense being incurred in respect of the conference, there shall be no liability attached to ISCP, AVBS, AAS, HBPRCA, or the Conference Organisers.

**Parking** - The Adelaide Convention Centre has two payable convenient onsite car parks, Riverbank and North Terrace (Please see page 10).

**Privacy** - Any information relevant to your attendance at the conference will be shared and used between ISCP, AVBS, AAS, HBPRCA and the conference organisers for the purposes of this conference. A list of delegates will be made available to conference delegates and sponsors.
Plaza Level

Main Reception
Access via stairs, escalator, lift or ramp

STATE OF THE HEART CONGRESS SPECIFIC ROOMS
COMMUNAL AREAS
REGATTAS BISTRO AND BAR

Riverbank Level

North Terrace, Adelaide South Australia 5000
Telephone: (618) 8210 6758
Cardiovascular disease kills one Australian every 12 minutes.¹

It affects one in six Australians (more than 3.7 million Australians), and prevents 1.4 million people from living a full life because of disability caused by the disease.¹

AstraZeneca continues to provide one of the broadest range of CV medicines to Australian patients including medications to lower cholesterol, reduce high blood pressure and reduce risk of secondary CV events after ACS.


LDL receptors are in a deadly bind.¹²

**KNOWN:** LDL receptors in the liver are essential for removing plasma LDL cholesterol.³

**DISCOVERED:** An endogenous protein called PCSK9 promotes degradation of the LDL receptor. Reduced numbers of LDL receptors on the surface of the hepatocyte increases plasma LDL cholesterol.¹²

**REVEALED:** Mutations of the PCSK9 gene have been shown to have an impact on LDL cholesterol levels and cardiovascular risk.⁴


Amgen Australia Pty Ltd, ABN 31 051 057 428. 123 Epping Road, North Ryde NSW 2113. AUS1845/AMG3073/Oct 2014.
## Programme at a Glance - Wednesday 26th November

### 10.30 – 11.30
**Commercialising Discoveries - Hall D**

- Chris Nave (Brandon Capital Partners)
- Elizabeth Bjork (AstraZeneca)
- John Hopwood (SAHMRI)
- Steve Wesselingh (SAHMRI)

### 11.30 – 12.00
**Coffee Break (Hall A)**

### 12.00 – 14.00

#### AAS - Hall D
**Nutrition and Lifestyle**

**Chairpersons:**
- Peter Clifton (Adelaide, Australia)
- Trevor Mori (Perth, Australia)

- Do omega-3s prevent the fetal origins of vascular disease?
  - Michael Skilton (Sydney, Australia)

- How good are lifestyle treatments in preventing atherosclerotic events?
  - David Colquhoun (Brisbane, Australia)

- The diet for the 21st century
  - Manny Noakes (Adelaide, Australia)

#### HBPRCA - RB 1
**Neural and Peripheral Mechanisms**

- Reinnervation of renal afferent and efferent nerves at 5½ and 11 months after catheter-based radio-frequency renal denervation in sheep
  - Clive May (Melbourne, Australia)

- Anti-hypertensive treatment and cerebral blood flow in human hypertension
  - Emma Hart (Bristol, UK)

- Baroreceptor function is preserved following field stimulation of carotid baroreceptors in normotensive and hypertensive rats
  - Zahra Kouchaki (Sydney, Australia)

- Role of the renal nerves in a conscious rabbit model of chronic kidney disease
  - Geoffrey Head (Melbourne, Australia)

- Contribution of the area postrema to the increased cardiac sympathetic nerve activity in ovine heart failure
  - Yonis Abukar (Melbourne, Australia)

- Dehydration associated with periodic water intake exacerbates hypertension and renal disease in male spontaneously hypertensive rats
  - Lucinda Hilliard (Melbourne, Australia)

#### AVBS - RB 2
**Thrombosis and Platelets**

**Chairpersons:**
- Michael Hickey (Melbourne, Australia)
- Robert Andrews (Melbourne, Australia)

- Biomechanical thrombosis: a distinct thrombotic mechanism linking intestinal ischemia to remote organ injury
  - Shaun Jackson (Sydney, Australia)

- Caspases render apoptosis immunologically silent by suppressing mtDNA-induced STING-mediated type I IFN production
  - Benjamin Kile (Melbourne, Australia)

- The shear excitement of platelets
  - Elizabeth Gardiner (Melbourne, Australia)

- Diabetes increases reticulated platelets due to enhanced proliferation and expansion of bone marrow megakaryocyte progenitors
  - Michael Kraakman (Melbourne, Australia)

- Molecular ultrasound imaging using platelet-targeted microbubbles: Diagnosis, monitoring and efficacy testing of thrombolytic drugs
  - Xiaowei Wang (Melbourne, Australia)

### 14.00 – 14.45
**Afternoon Break**

### 14.45 – 15.30
**China-Aus Vascular Biology/ASCEPT RB 3**

**Chairpersons:**
- Tracey Gaspari (Melbourne, Australia)
- Andrew Murphy (Melbourne, Australia)

- Macrophages as mediators of vessel remodelling in hypertension
  - Grant Drummond (Melbourne, Australia)

- Matrix metabolism and cardiovascular disease
  - Wei Kong (Beijing, China)

- Senescent endothelial cells show a unique anti-inflammatory phenotype
  - Jenny Gamble (Newtown, Australia)
### AAS - HALL D
#### DIABETES
**Chairpersons:**
- Terri Allen (Melbourne, Australia)
- Joanne Tan (Sydney, Australia)

**Atherosclerosis in diabetes: metabolic karma?**
- Merlin Thomas (Melbourne, Australia)

**Mechanisms of monocytes in obesity: Role of adipose tissue macrophages**
- Prabhakar Nagareddy (Kentucky, USA)

- Fibrous cap thickness of non-culprit plaques in diabetic and non-diabetic patients in response to LDL-c lowering therapy: insights from frequency-domain optical coherence tomography analysis
  - Yu Kataoka (Adelaide, Australia)

- Pancreatic β-cell specific deletion of ABCA1 and ABCG1 perturbs glucose metabolism and increases adiposity in mice due to suboptimal insulin production
  - Blake Cochran (Sydney, Australia)

- High density lipoproteins rescue diabetes-impaired angiogenesis via scavenger receptor class b type I
  - Joanne Tsui Ming Tan (Sydney, Australia)

- ApoA-I reduces inflammation in adipocytes by inhibiting TLR4-mediated inflammatory signalling pathways
  - Afroza Sultana – student finalist
  - (Sydney, Australia)

#### HBPRCA - RB 1
**STUDENT AND EARLY CAREER ORAL FINALISTS**

**Student finalists:**
- Randomized controlled trial on the effect of vitamin D supplementation on peripheral and central blood pressure, visit-to-visit blood pressure variability and aortic stiffness in older individuals
  - Panagiota Veloudi (Hobart, Tasmania)

- Cardiac actin-mysin cross-bridge dysregulation occurs early in the pathogenesis of type 2 diabetic cardiomyopathy
  - Mark Waddingham (Melbourne, Australia)

- Treatment with the Mas receptor agonist, AVE-0991, restores the normal regulation of arterial pressure in ACE2 deficient mice
  - Katrina Mirabito (Melbourne, Australia)

- Effects of Insulin Regulated Aminopeptidase (IRAP) Inhibition in angiotensin II-induced hypertension
  - Huey Wen Lee (Melbourne, Australia)

**Early Career Researcher Finalists:**
- Renal sympathetic innervation regulates blood pressure by actions on miR-181a and renin
  - Francine Marques (Ballarat, Australia)

- The contribution of orexin to the neurogenic hypertension in BPH/2J mice
  - Kristy Jackson (Melbourne, Australia)

- Effects of anti-hypertensive treatment on functional and structural components of large artery stiffness and retinal vessel diameters in a rodent model of type 1 diabetes
  - Yugeesh Lankadeva (Melbourne, Australia)

### AVBS - RB 2
#### IMMUNE MECHANISMS IN Atherosclerosis
**Chairpersons:**
- Grant Drummond (Melbourne, Australia)
- Len Kritharides (Sydney, Australia)

- Differential effects of B cell subsets in atherosclerosis
  - Alex Bobik (Melbourne, Australia)

- Monocyte production in diabetes and the contribution to atherosclerosis
  - Mary Kavurma (Sydney, Australia)

- The multiple roles of chemokines in atherosclerosis
  - Ying-Chih Chen (Melbourne, Australia)

### CHINA-AUS VASCULAR BIOLOGY/ASCEPT RB 3
**Chairpersons:**
- Barbara Kemp-Harper (Melbourne, Australia)
- Amanda Sampson (Melbourne, Australia)

**Vascular aging**
- Alex Chen (Changsha, China)

**High intraluminal pressure increases vascular inflammation**
- Jaye Chin-Dusting (Melbourne, Australia)

**The mechanism by which mechanical stress activates AT1 receptors**
- Yunzeng Zou (Shanghai, China)

### PLENARY SESSION - HALL D - Chairperson: Jaye Chin-Dusting (Melbourne, Australia)
**Colin I Johnston Lecture: The A to B of heart failure: not a strain for hypertension specialists**
- Tom Marwick (Hobart, Tasmania)

**Welcome reception/Posters and drinks**
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<th>Time</th>
<th>Session</th>
<th>Location</th>
<th>Chairpersons</th>
<th>Talks</th>
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<td>08.00 – 08.45</td>
<td><strong>PLENARY - HALL D</strong></td>
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<td>Meeting Welcome: Stephen Nicholls (Adelaide, Australia)</td>
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<td>ISCP President Welcome: George Dan (Bucharest, Romania)</td>
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<td><strong>Henry Neufeld Lecture:</strong> Energetic/inflammatory disturbances in heart disease: emerging therapeutic perspective John Horowitz (Adelaide, Australia)</td>
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<td>08.45 – 10.15</td>
<td><strong>ISCP - RB 3</strong></td>
<td>ISCP - RB 3</td>
<td>George Dan (Bucharest, Romania)</td>
<td>TREATING THE PATIENT WITH AFIB</td>
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<td>Margaret Arstall (Adelaide, Australia)</td>
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<td>Prevention of stroke in atrial fibrillation: are all oral drugs the same? Anti-arrhythmic drugs for atrial fibrillation Ablation or other non-pharmacological management of AF Cost effectiveness of oral anticoagulant drugs for stroke prevention in patients with non-valvular atrial fibrillation</td>
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<td>Antoni Martinez-Rubio (Sabadell, Spain)</td>
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<td>Prash Sanders (Adelaide, Australia)</td>
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<td>11.00 – 12.30</td>
<td><strong>ISCP/HBPRCA - HALL D</strong></td>
<td>ISCP/HBPRCA - HALL D</td>
<td>John McNeil (Melbourne, Australia)</td>
<td>TREATING HIGH BLOOD PRESSURE</td>
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<td>Arduino Mangoni (Adelaide, Australia)</td>
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<td>Guidelines for the management of high blood pressure</td>
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<td>Dietary salt and hypertension</td>
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<td>Bruce Neal (Sydney, Australia)</td>
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<td>Treating high blood pressure: combination therapy</td>
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<td>Thomas Kahan (Stockholm, Sweden)</td>
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<td>Oliver Soehnlein (Munich, Germany)</td>
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<td>Kevin Woollard (London, UK)</td>
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<td>Regulating the endothelium - interactions of regulatory T cells and vascular endothelial cells</td>
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<td>Epigenetic control of cardiac development and regeneration</td>
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<td>Exploiting allosteric and biased receptor signalling in cardiovascular disease</td>
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<td>A novel therapy for restoring heart function and rhythm</td>
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<td>CC-chemokine class inhibition attenuates inflammatory induced pathological angiogenesis whilst preserving ischaemia driven physiological angiogenesis</td>
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<td>Antéyah Ridiandries – student finalist (Sydney, Australia)</td>
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<td>Relationship of pericardial fat with biomarkers of inflammation and hemostasis, and cardiovascular disease: The Multi-Ethnic Study of Atherosclerosis</td>
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<td>Claudine Bonder (Adelaide, Australia)</td>
<td>Chairman: Claudine Bonder (Adelaide, Australia)</td>
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<td>ANGIOGENESIS AND LYMPHANGIOGENESIS</td>
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<td>Veronique Angeli (Singapore)</td>
<td>Chairman: Veronique Angeli (Singapore)</td>
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<td>Lymphatic vessels in atherogenesis</td>
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<td>Natasha Harvey (Adelaide, Australia)</td>
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<td>Regulation of vascular proteins by allosteric disulphide bonds</td>
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<td>Philip Hogg (Sydney, Australia)</td>
<td>Chairman: Philip Hogg (Sydney, Australia)</td>
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<td>Understanding the mechanisms by which GATA2 mutations cause primary lymphoedema</td>
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<td>Natasha Harvey (Adelaide, Australia)</td>
<td>Chairman: Natasha Harvey (Adelaide, Australia)</td>
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<td>Regulation of lymphangiogenesis in cancer</td>
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<td>Sophie Paquet-Fifield (Melbourne, Australia)</td>
<td>Chairman: Sophie Paquet-Fifield (Melbourne, Australia)</td>
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### PROGRAMME AT A GLANCE - Thursday 27th November

<table>
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<tr>
<td>12.30 – 13.15</td>
<td><strong>AAS AGM - RB 3</strong></td>
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<tr>
<td>12.30 – 13.30</td>
<td><strong>LUNCH</strong></td>
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</table>
| 13.30 – 14.00 | **PLENARY SESSION ISCP Invited Lecture - HALL D**  
Targeting inflammatory and lipid pathways for the treatment of cardiovascular disease  
Harvey White (Auckland, New Zealand) |
| 14.00 – 15.30 | **ISCP/AVBS - HALL D**  
TREATING PATIENTS WITH CORONARY DISEASE  
Chairpersons:  
Stephen Nicholls (Adelaide, Australia)  
Gemma Figtree (Sydney, Australia)  
Novel ways to inhibit platelets without bleeding complications  
Karlheinz Peter (Melbourne, Australia)  
Observational data / use of stabilised treatments  
Phil Aylward (Adelaide, Australia)  
Novel management of stable angina pectoris  
Juan Carlos Kaski (London, UK)  
Microcirculation in coronary artery disease  
Martin Ng (Sydney, Australia)  
Shear stress recruits the TRPV4 ion channel in acetylcholine-dependent vasodilatation  
William Darby (Melbourne, Australia) |
| 15.30 – 16.15 | **AFTERNOON BREAK**                                                 |
| 14.00 – 15.30 | **HBPRCA - RB 3**  
THE ROLE OF GENETICS IN HYPERTENSION  
Chairpersons:  
Stephen Harrap (Melbourne, Australia)  
Francine Marques (Ballarat, Australia)  
PVN Gαi2 subunit proteins – the key to a salt-resistant phenotype? – AHA award winner  
Richard Wainford (Boston, USA)  
The distribution of some single nucleotide polymorphisms of the renin-angiotensin system in Indigenous Australians  
Eugenie Lumbers (Newcastle, Australia)  
FOXO3 genotype increases lifespan by a major effect on cardiovascular mortality  
Brian Morris (Sydney, Australia)  
MicroRNAs mediate the protective effect of angiotensin converting enzyme inhibition (ACEI) on the heart in rat model of acute kidney injury  
Indrajiet Singh Rana (Ballarat, Australia)  
Genetic deficiency or pharmacological depletion of B cells prevents Angiotensin II-induced hypertension in mice  
Christopher Chan (Melbourne, Australia)  
A novel insertion somatic KCNJ5 mutation in an Australian patient with an aldosterone-producing adenoma  
Michael Stowasser (Brisbane, Australia) |
| 15.30 – 16.15 | **AAS - RB 1/2**  
OXIDATIVE STRESS  
Chairpersons:  
Judy de Haan (Melbourne, Australia)  
Shane Thomas (Sydney, Australia)  
The effects of dietary polyphenol ‘antioxidants’ on vascular function and blood pressure  
Kevin Croft (Perth, Australia)  
Bilirubin and atherosclerosis: could prevention of oxidative stress contribute to protection?  
Andrew Bulmer (Brisbane, Australia)  
Novel roles of heme oxygenase-1 in vascular protection  
Roland Stocker (Sydney, Australia)  
Lack of the antioxidant enzyme Glutathione Peroxidase-1 (GPx1) facilitates a pro-inflammatory and activated vascular endothelium: implications for endothelial dysfunction and atherosclerosis  
Judy de Haan (Melbourne, Australia)  
Neopterin is a direct marker of γ-interferon activation of macrophages and oxidative stress within human atherosclerotic plaque  
Steven Gieseg (Christchurch, New Zealand) |
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<tr>
<th>Time</th>
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<tr>
<td>16.15 – 17.45</td>
<td>ISCP/AAS - HALL D</td>
<td><strong>NOVEL AGENTS FOR DYSLIPIDAEMIA</strong>&lt;br&gt;“Supported by an unrestricted educational grant from Amgen Australia Pty Ltd”&lt;br&gt;&lt;br&gt;<strong>Chairpersons:</strong>&lt;br&gt;Stephen Nicholls (Adelaide, Australia)&lt;br&gt;Kerry-Anne Rye (Sydney, Australia)&lt;br&gt;&lt;br&gt;<strong>A critical look at novel LDL-lowering therapies</strong>&lt;br&gt;Len Kritharides (Sydney, Australia)&lt;br&gt;&lt;br&gt;<strong>Is there a future for CEPT inhibitors?</strong>&lt;br&gt;Philip Barter (Sydney, Australia)&lt;br&gt;&lt;br&gt;<strong>HDL-targeted therapies: progress, failures and future</strong>&lt;br&gt;Bronwyn Kingwell (Melbourne, Australia)&lt;br&gt;&lt;br&gt;Regression of coronary atherosclerosis in response to infusion of high-density lipoprotein mimetic agent CER-001 in patients with acute coronary syndrome&lt;br&gt;Jordan Andrews (Adelaide, Australia)</td>
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<td>17.45 – 18.45</td>
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<td><strong>HBPRCA AGM 16.45 – 17.45</strong></td>
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**Chairpersons:**<br>Jaye Chin-Dusting (Melbourne, Australia)<br><br>**Anatomical and physiological profiling to identify appropriate therapy for treatment resistant hypertension**<br>Amy Burchell (Bristol, UK) – BHS Award winner<br><br>Cost-effectiveness of angiotensin-converting enzyme inhibitor-based compared to thiazide diuretic-based treatment in an elderly hypertensive population considering diabetes as a major comorbidity<br>Enayet Chowdhury (Melbourne, Australia) |

**Chairpersons:**<br>Karlheinz Peter (Melbourne, Australia)<br>Peter Little (Melbourne, Australia)<br><br>**Bm1, a novel biomarker and regulator of endothelial forming cells**<br>Kate Parham (Adelaide, Australia)<br><br>Opposing effects of Nox isoforms in diabetes associated atherosclerosis<br>Stephen Gray (Melbourne, Australia)<br><br>Glycol-split heparin as a novel therapeutic for targeting myeloperoxidase-induced endothelial dysfunction<br>Enoch Chan (Sydney, Australia) |

**Award Presentations:**<br>AVBS Career Development Award Winner – Andrew Murphy (Melbourne, Australia)<br>AVBS Distinguished Researcher Award Winners – Len Kritharides and Wendy Jessup (Sydney, Australia)
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<th>08.30 – 10.00</th>
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<th>AAS - RB 1/2</th>
<th>AVBS/HBP RACA - HALL D</th>
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<tr>
<td><strong>NEW APPROACHES TO TREATMENT OF DIABETES AND HEART DISEASE</strong></td>
<td><strong>NEW INVESTIGATOR SESSION</strong></td>
<td><strong>CLINICAL INSIGHTS INTO VASCULAR DISEASE</strong></td>
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<td><strong>Chairpersons:</strong></td>
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<td>Karin Jandeleit-Dahm (Melbourne, Australia)</td>
<td>Heather Medbury (Sydney, Australia)</td>
<td>Kevin Croft (Perth, Australia)</td>
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<td>Steve Stranks (Adelaide, Australia)</td>
<td>Steven Gieseg (Christchurch, New Zealand)</td>
<td>James Sharman (Hobart, Tasmania)</td>
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<td>Imaging / MRI</td>
<td>High-density lipoprotein inhibits human M1 macrophage polarisation through the redistribution of caveolin-1</td>
<td>Renal denervation after Symplicity HTN-3: where to from here?</td>
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<td>Tom Marwick (Hobart, Tasmania)</td>
<td>Man Lee (Melbourne, Australia)</td>
<td>Markus Schlaich (Perth, Australia)</td>
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<td><strong>New and old drugs in diabetes and heart failure</strong></td>
<td>Deficiency of Nox4-derived ROS promotes a pro-atherogenic phenotype in mouse aortic smooth muscle cells exposed to high glucose</td>
<td><strong>Does diet have a role in secondary prevention?</strong></td>
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<td>Louise Burrell (Melbourne, Australia)</td>
<td>Elyse Marco (Melbourne, Australia)</td>
<td>Peter Clifton (Adelaide, Australia)</td>
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<tr>
<td><strong>New and old drugs in diabetes and atherosclerosis</strong></td>
<td>Apolipoprotein A-I increases glucose uptake in skeletal muscle and improves glycaemic control in db/db mice</td>
<td>Ten year legacy effects of baseline blood pressure treatment naivety in the Second Australian National Blood Pressure study</td>
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<td>Mark Cooper (Melbourne, Australia)</td>
<td>Shudi Tang (Sydney, Australia)</td>
<td>Mark Nelson (Hobart, Tasmania)</td>
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<td><strong>Treatment-free hypertension</strong></td>
<td>ApoA-I suppresses neointimal hyperplasia following stent deployment and modulates neointimal cellular phenotype</td>
<td>Prediction of heart failure by serum amino-terminal-pro-B-type natriuretic peptide (NT-proBNP): an interim analysis of the Screening Evaluation of the Evolution of New Heart Failure (SCREEN-HF) study</td>
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<td>Charlotte Mills (London, UK)</td>
<td>Laura Vanags (Sydney, Australia)</td>
<td>Duncan Campbell (Melbourne, Australia)</td>
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<td>Impact of obesity and insulin resistance on serum dihomoγ-linolenic acid</td>
<td>A novel survival pathway of erythropoietin in protection of neonatal myocytes from cell death during hypoxia/reperfusion injury</td>
<td>ASPREE (ASPIrin in Reducing Events in the Elderly): a progress report</td>
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<td>Morihiro Matsuda (Osaka, Japan)</td>
<td>Asiya Allaudeen – student finalist (Chennai, India)</td>
<td>Robyn Woods (Melbourne, Australia)</td>
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<td>Expression profile of adhesion and chemokine markers on monocyte subsets and their correlation with atherosclerotic risk</td>
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<td>Vyoma Patel – student finalist (Sydney, Australia)</td>
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<td>Intrinsc cholesterol handling capacities of human macrophages derived from different monocyte subsets</td>
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<td>Annas Al-sharea – student finalist (Melbourne, Australia)</td>
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<td>Distribution of M2 macrophages within major and subregional areas of the atherosclerotic plaque</td>
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<td>Virginia James – student finalist (Sydney, Australia)</td>
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<td><strong>MORNING BREAK</strong></td>
<td><strong>PLENARY SESSION - HALL D</strong></td>
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<td>Austin Doyle Lecture: Everything you wanted to know about cosmology, but were afraid to ask</td>
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<td>Brian Schmidt (ANU Nobel Laureate)</td>
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<td>11.15 – 12.45</td>
<td>ISCP - RB 3</td>
<td>ISCP - RB 3</td>
<td>Dayi Hu (Beijing, China) Watt Wendy Keech (Adelaide, Australia)</td>
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<td>Aborginal population Alex Brown (Adelaide, Australia)</td>
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<td>Renal failure / reducing risk Vlado Perkovic (Sydney, Australia)</td>
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<td>Statin use for &quot;primary prevention&quot; doubles with the new &quot;ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk&quot;: comparison of two American guidelines Cem Barcin (Ankara, Turkey)</td>
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<td>FXDY1 plays a protective role against myocardial fibrosis Jian Li (Sydney, Australia)</td>
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<td>13.30 – 14.00</td>
<td>PLENARY SESSION - HALL D - Chairperson: Jaye Chin-Dusting (Melbourne, Australia)</td>
<td>HALL D</td>
<td>Rhian Touyz (UK)</td>
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<td>14.00 – 14.30</td>
<td>ISCP - RB 3</td>
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<td>TARGETING THE OBESITY EPIDEMIC</td>
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<td>Chairpersons: Steve Wesselingh (Adelaide, Australia) Manny Noakes (Adelaide, Australia)</td>
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<td>Pharmacotherapy for obesity: is there any hope? Gary Wittert (Adelaide, Australia)</td>
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<td>New approaches for obesity: effects on cardiovascular outcomes Stephen Nicholls (Adelaide, Australia)</td>
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<td>Sleep apnoea / CV risk Nick Antic (Adelaide, Australia)</td>
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<td>14.30 – 16.00</td>
<td>AVBS/AAS/HBPRCA - HALL D</td>
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<td>THE IMMUNE RESPONSE IN CARDIOVASCULAR DISEASE</td>
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<td>Chairpersons: Andrew Murphy (Melbourne, Australia) John Horowitz (Adelaide, Australia)</td>
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<td>Enhanced myeloid cell glucose utilization in atherosclerosis Laurent Yvan-Chavet (Nice, France)</td>
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<td>GM-CSF activates cardiac inflammation during Kawasaki disease Ian Wicks (Melbourne, Australia)</td>
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<td>Mechanisms of systemic immune suppression Connie Wong (Melbourne, Australia)</td>
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<td>16.00 – 16.30</td>
<td>AAS - RB 1/2</td>
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<td>LIPIDS</td>
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<td>Chairpersons: David Sullivan (Sydney, Australia) Fatiha Tabet (Sydney, Australia)</td>
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<td>20 years on from 4S: have we sorted out LDL? Stephen Nicholls (Adelaide, Australia)</td>
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<td>How HIV, or parts or thereof, causes dyslipidaemia and atherosclerosis Dimitri Svirdov (Melbourne, Australia)</td>
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<td>HDL-small RNA intercellular communication and atherosclerosis Kasey Vickers (Nashville, US)</td>
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<td>Cellular cholesterol homeostasis is altered in murine models of rheumatoid arthritis Dragana Dragoljevic – student finalist (Melbourne, Australia)</td>
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<td>Lipoprotein(a) stimulates ABCA1 in the liver cells via Scavenger Receptor-B1 Monika Sharma – student finalist (Dunedin, New Zealand)</td>
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TREATING HEART FAILURE

16.30 – 17.45
ISCP - RB 1/2

TREATING HEART FAILURE

Chairpersons:
John Beltrame (Adelaide, Australia)
Carmine Depasquale (Adelaide, Australia)

RAAS inhibition and heart failure: the end or the beginning of a new era?
Felipe Martinez (Cordoba, Argentina)

HEFREF (HF with reduced EF)
John Atherton (Brisbane, Australia)

Novel interventions for heart failure
Stephen Worthley (Adelaide, Australia)

AVBS/AAS - HALL D

ANEURYSMAL VASCULAR DISEASE

Chairpersons:
Alex Bobik (Melbourne, Australia)
Mary Kavurma (Sydney, Australia)

Inflammatory mechanisms in abdominal aortic aneurysm: translating findings from mice to patients
Joseph Moloney (Townsville, Australia)

Potential mechanisms and therapies for abdominal aortic aneurysm
Robert Gibson (Melbourne, Australia)

Effects of blood pressure lowering on cardiovascular risk according to baseline body mass index: a meta-analysis of randomized trials
Bruce Neal (Sydney, Australia)

Inflammatory mechanisms in abdominal aortic aneurysm
Keith Channon (Oxford, UK)

Thrombospondin-1 deficiency promotes maladaptive extracellular matrix remodeling in abdominal aortic aneurysm
Shalini Krishnan (Melbourne, Australia)

HBPRCA FREE COMMUNICATIONS - RB 3

Chairpersons:
Rob Widdop (Melbourne, Australia)
Brian Morris (Sydney, Australia)

Effects of blood pressure lowering on cardiovascular risk according to baseline body mass index: a meta-analysis of randomized trials
Bruce Neal (Sydney, Australia)

Angiotensin converting enzyme 2 deficiency promotes aortic aneurysm formation and rupture in apolipoprotein E-deficient mice
Bruce Neal (Sydney, Australia)

Investigating the role of B cells in the vascular wall during angiotensin II-induced hypertension in mice
Yutang Wang (Ballarat, Australia)

Effect of a high fat diet and/or chronic stress on cardiovascular function in mice
Wendy Muller (Melbourne, Australia)

Inflammation activity is essential for deoxycorticosterone acetate/salt-induced hypertension in mice
Eleanor Gould (Melbourne, Australia)

Inflammasome activity is essential for deoxycorticosterone acetate/salt-induced hypertension in mice
Shalini Krishnan (Melbourne, Australia)
State of the Heart 2014 would like to thank the following Corporate members and Sponsors for their support at this event.

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Bronze Sponsor

Others
### Commercialising Discoveries

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<tr>
<td></td>
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<td>Chris Nave</td>
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<td>Steve Wesselingh</td>
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<td>John Hopwood</td>
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<td>Elizabeth Bjork</td>
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<td>Stephen Nicholls</td>
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### AAS Session - Nutrition and Lifestyle

**Chairpersons:** Peter Clifton, Trevor Mori

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<tr>
<td>12.00</td>
<td>Michael Skilton</td>
<td>Do omega-3s prevent the fetal origins of vascular disease?</td>
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<tr>
<td>12.20</td>
<td>David Colquhoun</td>
<td>How good are lifestyle treatments in preventing atherosclerotic events?</td>
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<td>12.40</td>
<td>Manny Noakes</td>
<td>The diet for the 21st Century</td>
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<tr>
<td>13.00</td>
<td>Anmar Khan</td>
<td>The effect of dietary weight loss and exercise on the plasma and lipoprotein lipidomes</td>
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<tr>
<td>13.15</td>
<td>Natalie Blanch (Student finalist)</td>
<td>Effects of increased potassium and sodium on endothelial and vascular function</td>
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<td>13.30</td>
<td>Trevor Mori</td>
<td>Resolution of inflammation is impaired in the metabolic syndrome</td>
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<tr>
<td>13.45</td>
<td>Dick Chan</td>
<td>Relationships between proprotein convertase subtilisin/kexin type 9, apolipoprotein C-III and plasma apolipoprotein B-48 transport in obese subjects: a stable isotope study in the postprandial state</td>
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### HBPRCA Session - Neural and Peripheral Mechanisms

**Chairpersons:** Mark Nelson, Amanda Sampson

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<tr>
<td>12.00</td>
<td>Clive May</td>
<td>Reinnervation of Renal Afferent and Efferent Nerves at 5 ½ and 11 Months after Catheter-based Radio-frequency Renal Denervation in Sheep</td>
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<td>12.15</td>
<td>Emma Hart</td>
<td>Anti-hypertensive treatment and cerebral blood flow in human hypertension</td>
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<td>12.30</td>
<td>Zahra Kouchaki</td>
<td>Baroreceptor function is preserved following field stimulation of carotid baroreceptors in normotensive and hypertensive rats</td>
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<tr>
<td>12.45</td>
<td>Geoffrey Head</td>
<td>Role of the renal nerves in a conscious rabbit model of chronic kidney disease</td>
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<tr>
<td>13.00</td>
<td>Brad Broughton</td>
<td>G protein-coupled estrogen receptor signaling improves stroke outcome in female mice</td>
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<tr>
<td>13.15</td>
<td>Yonis Abukar</td>
<td>Contribution of the area postrema to the increased cardiac sympathetic nerve activity in ovine heart failure</td>
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<tr>
<td>13.30</td>
<td>Lucinda Hilliard</td>
<td>Dehydration associated with periodic water intake exacerbates hypertension and renal disease in male spontaneously hypertensive rats</td>
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<tr>
<td>13.45</td>
<td>Shenpeng Zhang</td>
<td>Th2-promoting cytokine treatment limits brain injury after cerebral ischemia in Th1-dominant mice</td>
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### AVBS Session - Thrombosis and Platelets

**Chairpersons:** Michael Hickey, Robert Andrews

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<th>Time</th>
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<tbody>
<tr>
<td>12.00</td>
<td>Shaun Jackson</td>
<td>Biomechanical Thrombosis: A Distinct Thrombotic Mechanism Linking Intestinal Ischemia to Remote Organ Injury</td>
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<tr>
<td>12.30</td>
<td>Benjamin Kile</td>
<td>Caspases render apoptosis immunologically silent by suppressing mtDNA-induced STING-mediated type I IFN production</td>
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<tr>
<td>13.00</td>
<td>Elizabeth Gardiner</td>
<td>The shear excitement of platelets</td>
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<tr>
<td>13.30</td>
<td>Michael Kraakman</td>
<td>Diabetes Increases Reticulated Platelets due to Enhanced Proliferation and Expansion of Bone Marrow Megakaryocyte Progenitors</td>
</tr>
<tr>
<td>13.45</td>
<td>Xiaowei Wang</td>
<td>Molecular ultrasound imaging using platelet-targeted microbubbles: Diagnosis, monitoring and efficacy testing of thrombolytic drugs</td>
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### China-Aus Vascular Biology / ASCEPT

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>12.00</td>
<td>Grant Drummond</td>
<td>Macrophages as mediators of vessel remodelling in hypertension</td>
</tr>
<tr>
<td>12.40</td>
<td>Wei Kong</td>
<td>Matrix metabolism and cardiovascular disease</td>
</tr>
<tr>
<td>13.20</td>
<td>Jenny Gamble</td>
<td>Senescent endothelial cells show an unique anti-inflammatory phenotype</td>
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### AAS Session - Diabetes

**Chairpersons:** Terri Allen, Joanne Tan

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<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>14.45</td>
<td>Merlin Thomas</td>
<td>Atherosclerosis in diabetes: metabolic karma?</td>
</tr>
<tr>
<td>15.15</td>
<td>Prabhakar Nagareddy</td>
<td>Mechanisms of monocytosis in obesity: role of Adipose tissue macrophages</td>
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Wednesday 26th November
<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>14.45</td>
<td>Yu Kataoka</td>
<td>Fibrous Cap Thickness of Non-culprit Plaques in Diabetic and Non-diabetic Patients in Response to LDL-C Lowering Therapy: Insights from Frequency-Domain Optical Coherence Tomography Analysis</td>
</tr>
<tr>
<td>15.50</td>
<td>Blake Cochran</td>
<td>Pancreatic B-cell specific deletion of ABCA1 and ABCG1 perturbs glucose metabolism and increases adiposity in mice due to suboptimal insulin production</td>
</tr>
<tr>
<td>15.40</td>
<td>Joanne Tan</td>
<td>High Density Lipoproteins Rescue Diabetes-Impaired Angiogenesis via Scavenger Receptor Class B Type I</td>
</tr>
<tr>
<td>16.45</td>
<td>Afroza Sultana</td>
<td>ApoE-I reduces inflammation in adipocytes by inhibiting TLR4-mediated inflammatory signalling pathways</td>
</tr>
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### 14.45 - 16.45 HBPRCA - Student and Early Career Oral Finalists (RB 1)

#### Chairpersons
- Kate Denton
- Jim Sharman

#### Student Finalists

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<tr>
<th>Time</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>14.45</td>
<td>Panagiota Veloudi</td>
<td>Randomized controlled trial on the effect of vitamin D supplementation on peripheral and central blood pressure, visit-to-visit blood pressure variability and aortic stiffness in older individuals</td>
</tr>
<tr>
<td>15.00</td>
<td>Mark Waddingham</td>
<td>Cardiac actin-myosin cross-bridge dysregulation occurs early in the pathogenesis of type 2 diabetic cardiomyopathy</td>
</tr>
<tr>
<td>15.15</td>
<td>Katrina Mirabito</td>
<td>Treatment with the Mas receptor agonist, AVE-0991, restores the normal regulation of arterial pressure in ACE2 deficient mice</td>
</tr>
<tr>
<td>15.30</td>
<td>Huey Wen Lee</td>
<td>Effects of Insulin Regulated Aminopeptidase (IRAP) Inhibition in angiotensin II-induced hypertension</td>
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### 14.45 - 16.45 AVBS Session - Immune Mechanisms in Atherosclerosis (RB 2)

#### Chairpersons
- Grant Drummond
- Len Kritharides

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<tr>
<th>Time</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>14.45</td>
<td>Alex Bobik</td>
<td>Differential Effects of B Cell Subsets in Atherosclerosis</td>
</tr>
<tr>
<td>15.15</td>
<td>Andrew Murphy</td>
<td>Monocyte production in diabetes and the contribution to atherosclerosis</td>
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<tr>
<td>14.45</td>
<td>Mary Kavurma</td>
<td>On the TRAIL of atherosclerosis</td>
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<tr>
<td>16.05</td>
<td>Christina Bursill</td>
<td>The multiple roles of chemokines in atherosclerosis</td>
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<tr>
<td>16.25</td>
<td>Yung-Chih Chen</td>
<td>Therapeutic role of TLR9 agonist in atherosclerosis</td>
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### 14.45 - 16.45 China-Aus Vascular Biology / ASCEPT (RB 3)

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>12.00</td>
<td>Alex Chen</td>
<td>Vascular aging</td>
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<tr>
<td>12.40</td>
<td>Jaye Chin-Dusting</td>
<td>High intraluminal pressure increases vascular inflammation</td>
</tr>
<tr>
<td>13.20</td>
<td>Yunzeng Zou</td>
<td>The mechanism by which mechanical stress activates AT1 receptors</td>
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### 16.45 - 17.15 PLENARY SESSION - COLIN I JOHNSTON LECTURE (HALL D)

#### Chairperson
- Jaye Chin-Dusting

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<tr>
<th>Time</th>
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<tbody>
<tr>
<td>16.45</td>
<td>Tom Marwick</td>
<td>The A to B of heart failure: not a strain for hypertension specialists</td>
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### 17.30 MODERATED POSTER SESSION (HALLS J/K)

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<thead>
<tr>
<th>Time</th>
<th>Poster</th>
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<tbody>
<tr>
<td>17.30</td>
<td>Alexander Chalmers</td>
<td>Describing the efficacy of HDL in early atherosclerosis via mathematical modelling</td>
</tr>
<tr>
<td>17.35</td>
<td>Melissa Francis</td>
<td>Familial hypercholesterolaemia and cardiovascular outcomes</td>
</tr>
<tr>
<td>17.40</td>
<td>Rasheed Humaira</td>
<td>Association analysis of apolipoprotein B and very low-density lipoprotein with hyperuricemia and gout</td>
</tr>
<tr>
<td>17.45</td>
<td>Edward Marks</td>
<td>sFLT-1, a robust biomarker for diagnosis and prognosis in heart disease</td>
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<tr>
<td>17.50</td>
<td>Muhammad Mohtar</td>
<td>Thymoquinine and Nigella sativa inhibit in vitro lipopolysaccharide-induced adhesion of monocytes to human coronary artery endothelial cells</td>
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<tr>
<td>17.55</td>
<td>Kristina Petersen</td>
<td>The dietary predictors of pulse wave velocity in a cohort with diabetes</td>
</tr>
<tr>
<td>18.00</td>
<td>Francine Petrides</td>
<td>Expression of LDR and inhibition of PCSK9 with Alirocumab in heterozygous and homozygous familial hypercholesterolemic patients</td>
</tr>
<tr>
<td>18.05</td>
<td>Aliki Rasmiena</td>
<td>Attenuation of atherosclerosis by plasmalogen enrichment: an animal study</td>
</tr>
<tr>
<td>17.30</td>
<td>Megan Evans</td>
<td>Acute administration of human amnion epithelial cells reduces infarct volume and splenic atrophy following transient cerebral ischemia in mice</td>
</tr>
<tr>
<td>17.35</td>
<td>Remi Goupil</td>
<td>Does concomitant autonomous adrenal cortisol overproduction have the potential to confound the interpretation of adrenal venous sampling in primary aldosteronism?</td>
</tr>
<tr>
<td>17.40</td>
<td>Susan Morton</td>
<td>Vascular endothelial CaX40 contributes to activity-dependent blood pressure regulation</td>
</tr>
<tr>
<td>17.45</td>
<td>Nugeogadage Ranasinghe</td>
<td>Targeting inflammation in the brain during angiotensin II-induced hypertension</td>
</tr>
<tr>
<td>17.50</td>
<td>Amanda Sampson</td>
<td>Pressure-induced T cell homing in the aorta</td>
</tr>
<tr>
<td>17.55</td>
<td>Richard Schlegel</td>
<td>Maternal hypomagnesaemia does not cause programmed changes to cardiovascular physiology in adult offspring</td>
</tr>
<tr>
<td>18.00</td>
<td>Martin Schultz</td>
<td>Arterial impedance mismatching fails to explain central pressure augmentation: aortic reservoir function may be the prevailing factor</td>
</tr>
<tr>
<td>18.05</td>
<td>Isabella Tan</td>
<td>Augmentation index differs in paced subjects with different pacing modalities, independent of heart rate and blood pressure</td>
</tr>
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</table>
01: DO OMEGA-3S PREVENT THE FETAL ORIGINS OF VASCULAR DISEASE?

Skilton, Michael
Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, University of Sydney, Sydney, Australia

Physical evidence consistent with the earliest stages of atherosclerosis is present in the aorta during fetal life. The extent and severity of these earliest “lesions” are increased in those affected by impaired fetal growth, for which there is also strong evidence for associations with increased arterial wall thickness and impaired endothelial function during childhood, and risk of heart disease in adulthood. There is currently no widely accepted strategy through which to prevent the association of impaired fetal growth with atherosclerotic vascular disease. Emerging evidence suggests that dietary omega-3 fatty acids may be the first such preventive strategy, as they are associated with improved haemodynamic and vascular profile in children and adults who were born with impaired fetal growth. Putative mechanisms and implications for guidelines and practice will be discussed.

02: HOW GOOD ARE LIFESTYLE TREATMENTS IN PREVENTING ATHEROSCLEROTIC EVENTS?

Colquhoun, David
Brisbane, Australia

A healthy lifestyle is associated with low risk of atherosclerotic events – acute myocardial infarction and stroke. Five lifestyle factors - exercise, normal weight, small intake of alcohol, a Mediterranean type diet and not smoking explain two-thirds to three-quarters of incident coronary heart disease over 20 years. Lifestyle risk factors for stroke include high salt intake. For secondary prevention there are not many randomized control trials. For secondary prevention after myocardial infarction the stand out lifestyle trial is the Lyon Diet trial. A Mediterranean type diet compared an AHA step 2 diet is associated with 30 – 50% reduction in total mortality and CHD events.

Lifestyle measures are associated with decrease in cholesterol, triglycerides, hypertension and obesity. The relative and absolute risk reduction in cardiovascular events over a 5 year period or lifetime is of similar magnitude to drugs. More emphasis needs to be placed on the simple cost effective measures.

03: THE DIET FOR THE 21ST CENTURY

Noakes, Manny
Nutrition and Health, CSIRO

The 21st century will be characterised by a growing global population and growing chronic disease but hungry for personalised healthcare. By 2050, 22% of the World population will be over 60 years of age (29% in Australia). At the same time, the explosion in communication and information technology (ICT) as well as knowledge of the functions of the human genome, and the role of genetic modification will transform our diets and the foods we eat from what we know them today. Examples of this include decreases in fish stocks and the transformation of plants to produce long chain omega 3 fatty acids. New sources of food will abound. Oils and proteins extracted from novel plants will form substrates for new products which may be printed or extruded. The critical need to reduce waste in the food sector will generate biorefineries which transform food waste into edible components be they from animal or vegetable sources. These trends will create challenges in population dietary recommendations but ICT will allow individuals to personalise their diets according to their nutritional needs and their genetic and epigenetic profiles.

04: THE EFFECT OF DIETARY WEIGHT LOSS AND EXERCISE ON THE PLASMA AND LIPOPROTEIN LIPIDOMES

Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia;

Background and Aims: Metabolic syndrome (MetS) is associated with an increased risk of type 2 diabetes (T2D) and cardiovascular disease (CVD), due in part to the altered lipoprotein composition and metabolism. Changing lifestyle behaviours such as diet and physical activity are recommended as a first-line treatment to delay onset or prevent progression of T2D and CVD. However, the relationship between lifestyle changes, plasma lipids and disease risk is incompletely understood. In this study we have characterized the changes in the plasma and lipoprotein lipidomes resulting from dietary weight loss and exercise in MetS.

Methods: MetS participants (n=95) were matched for age and sex with a group of healthy controls (n=40). Subsets of the MetS group underwent 12 weeks of dietary weight loss (n=19) or dietary weight loss with exercise (n=17). Very low-density, low-density and high-density lipoproteins were isolated using sequential ultracentrifugation. Lipids (334 species) were analysed in plasma and lipoprotein by liquid chromatography-tandem mass spectrometry. Differences between MetS and control and the effect of treatment were identified using student t-tests and paired t-tests, respectively (p <0.05, corrected for multiple comparisons by the method of Benjamini-Hochberg). Hypergeometric analyses
were used to assess the effects of treatment on the MetS lipid profile.

**Results:** MetS participants had elevated plasma levels of di- and triacylglycerol and cholesterol esters, whereas, di- and trihexosylceramide, sphingomyelin and the lyso-, alkyl- and alkenylphospholipid lipid classes were lower compared to the control group. Following weight loss, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol decreased significantly. In contrast, weight loss combined with exercise resulted in most of the same changes in addition to decreases in ceramide, cholesteryl ester as well as di- and triacylglycerols. Of 211 lipid species associated with MetS, 40 changed significantly toward healthy group following weight loss, whereas, 81 lipids, changed significantly after weight loss with exercise. Subsequent analysis of the lipoprotein fractions showed a similar trend with the greatest shift of the lipid profiles toward the control group observed in the weight loss with exercise group.

**Conclusions:** The lipidomic composition of plasma and lipoproteins are altered in MetS. Lifestyle interventions altered the lipid profiles of plasma and lipoproteins towards the control group, with weight loss and exercise showing the greatest effect.

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**05: EFFECTS OF INCREASED POTASSIUM AND SODIUM ON ENDOTHELIAL AND VASCULAR FUNCTION**

**Blanch N, Petersen KS, Clifton PM, Keogh JB**

School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia

**Background and Aims:** Increased potassium intake has been related to improved endothelial function. High sodium intake is known to impair endothelial function. The effect of increasing potassium in the presence of high dietary sodium is not known. The aim was to determine the effect of increased potassium and increased sodium on post prandial endothelial function, as assessed by flow mediated dilatation (FMD), low flow mediated constriction (LFMC) and the chemical mediators endothelin-1, e-selectin and ICAM.

**Methods:** Healthy, normotensive volunteers (n=39, age 38±16 and BMI 23.1±2.9) received 3 meals on three separate occasions in a randomised order with either 3.1mmol potassium and 65mmol sodium (LKHNa), 38mmol potassium and 65mmol sodium (HKHNa) or (LKLNa) with 5.5mmol sodium and 3.1mmol potassium (control) . FMD, LFMC, pulse wave velocity (PWV), BP and serum samples were measured fasting and at 30, 60, 90 and 120 minutes after the meal. Repeated-measures ANOVA and paired t-tests were used to assess the effects of the meal type on the dependent variables over time.

**Results:** The addition of potassium (HKHNa) attenuated the post meal decrease in FMD over time when compared to the high sodium meal and control meals (p=0.000 meal effect; p=0.057 meal x time interaction). FMD was lower following the LKHNa meal when compared to the HKHNa meal at 30 minutes (-3.58±0.85%, p=0.000). LFMC was lower in the HKHNa group and higher in the HKHNa group when compared to LKLNa (p=0.000 for meal effect). Serum potassium was increased following the HKHNa meal when compared to the LKLNa and LKLNa meals (p=0.000 meal effect; p=0.000 meal x time interaction) with a maximum increase of 1.1±0.56mmol at 90 minutes. Serum sodium was increased following the HKHNa and HKHNa meals when compared to the LKLNa meal (p=0.000 meal effect; p=0.000 meal x time interaction) with a maximum increase of 1.2±0.99mmol at 60 minutes. There was a significant meal (p=0.011) and meal x time interaction (p=0.019) for serum endothelin-1 with a maximum reduction following the HKHNa at 120 minutes (-0.25±0.07pg/ml). ICAM-1 had a significant meal x time interaction for (p=0.002) in favour of the low Na meal. There were no significant differences in PWV, BP or e-selectin between treatments.

**Conclusions:** The addition of potassium to a high sodium meal attenuates the post meal reduction in endothelial function as assessed by FMD. Increases in sodium and potassium do not affect PWV or BP in the postprandial state.

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**06: RESOLUTION OF INFLAMMATION IS IMPAIRED IN THE METABOLIC SYNDROME**

**Mori TA, Mas E, Croft KD, Phillips M, Barden A**

University of Western Australia, School of Medicine & Pharmacology, Perth, Australia

**Background:** The metabolic syndrome (MetS) is associated with a chronic low-grade inflammatory state. The ability to resolve inflammation can affect immune responses and is an active process driven by specialised resorbing lipid mediators (SPM) derived from n-3 fatty acids (n-3FA).

**Aim:** To examine the effect of n-3FA supplements and aspirin on SPM in volunteers with the MetS and controls.

**Methods:** 21 controls and 22 MetS volunteers entered a 4 week study taking n-3FA (2.4g/day, 35% EPA + 25% DHA). After 3 weeks, aspirin (300mg/day) was consumed in addition to the n-3FA. Blood was collected at baseline, and after 3 and 4 weeks for measurement of SPM including 18-HEPE, E-series resolvins, 17-HDHA, D-series resolvins, 14-HDHA and MaR-1 by LCMSMS.

**Results:** At baseline concentrations of E- and D- series resolvins and their upstream precursors 18-HEPE, E-series resolvins, 17-HDHA, D-series resolvins, 14-HDHA and MaR-1 by LCMSMS.

**Conclusions:** Reduced levels SPM precursors in the MetS after n-3FA supplementation suggests that resolution of inflammation may be impaired affecting the ability to mount an appropriate immune response to infection.
**07: RELATIONSHIPS BETWEEN PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9, APOLIPROPROTEIN C-III AND PLASMA APOLIPROPROTEIN B-48 TRANSPORT IN OBESE SUBJECTS: A STABLE ISOTOPE STUDY IN THE POSTPRANDIAL STATE**

**Chan DC, Wong A, Pang J, Barrett PHR, Watts GF**  
University of Western Australia

**Background:** Postprandial lipaemia, due to elevated plasma apolipoprotein (apo) B-48 concentrations, contributes to increased cardiovascular risk in obesity. Proprotein convertase subtilisin/kexin type 9 (PCSK9) and apoC-III may play a role in regulating apoB-48 metabolism. We investigated the associations between plasma PCSK9 and apoC-III concentrations and the kinetics of apoB-48 in obese subjects.

**Methods:** Seventeen obese subjects were given an oral fat load. ApoB-48 tracer/traccee ratios were measured after intravenous d3-leucine administration using gas chromatography-mass spectrometry. Kinetic parameters, including secretion and fractional catabolic rates (FCR), were derived using a multi-compartmental model.

**Results:** Plasma PCSK9 and apoC-III concentrations were significantly and positively (P<0.05 in all) associated with the total area-under-curve (AUC) and incremental AUC for apoB-48, and inversely with apoB-48 FCR. Plasma PCSK9 and apoC-III concentrations were not correlated (P>0.05 in all) with basal secretion or number of apoB-48 secreted over the postprandial period. In stepwise regression analysis, plasma PCSK9 was the best predictor of the total and incremental AUCs for plasma apoB-48, and the FCR of apoB-48. The association between plasma PCSK9 and apoC-III and apoB-48 FCR remained significant (P<0.05 in all) after adjusting for age, homeostasis model assessment score (HOMA), hepatic lipase or lipoprotein lipase. In a multiple regression model, 31% of variance in apoB-48 FCR was accounted for by plasma PCSK9 and apoC-III concentrations (adjusted R2 =0.306, P<0.05). However, their associations with TRL-apoB-48 FCR were not independent of each other.

**Conclusion:** Our results suggest that the catabolism of TRL-apoB-48 in the postprandial state may be coordinated by PCSK9 and apoC-III in obese individuals.

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**HBPRCA Session - Neural and Peripheral Mechanisms**

**08: REINNERVATION OF RENAL AFFERENT AND EFFERENT NERVES AT 5 ½ AND 11 MONTHS AFTER CATHETER-BASED RADIO-FREQUENCY RENAL DENERVATION IN SHEEP**

**May CNa, Nishi E, Yao ST, Ranchandra R, Lambert GW, Schlaich MP, Booth LSa**  
aFlorey Institute of Neuroscience and Mental Health, Melbourne, Victoria, Australia; bDepartment of Physiology, Cardiovascular Division, Federal University of São Paulo, São Paulo, Brazil; cBaker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia

**Background:** Catheter-based renal denervation has been shown to reduce blood pressure and renal norepinephrine spillover in human resistant hypertension. The effects of this procedure on afferent sensory and efferent sympathetic renal nerves, and the subsequent degree of reinnervation, have not been investigated.

**Aims:** To examine the level of functional and anatomical reinnervation at 5.5 and 11 months after renal denervation using the Symplicity Flex catheter in normotensive, anaesthetized sheep.

**Methods:** The responses of mean arterial pressure, heart rate and renal blood flow to electrical stimulation of the renal nerve were determined in anaesthetized control non-denervated sheep (n=6), immediately after renal denervation (n=6) and at 5.5 (n=5) and 11 (n=5) months after renal denervation.

**Results:** In control sheep, electrical stimulation of intact renal nerves increased arterial pressure from 99±3 to 107±3 mmHg (afferent response) and reduced renal blood flow (198±16 to 85±20 mL/min) (efferent response). Immediately after denervation, renal sympathetic nerve activity (RSNA) was absent and the responses to electrical stimulation were abolished. At 11 months post-denervation, RSNA and the responses to electrical stimulation were at normal levels. Immunohistochemical staining for renal afferent (tyrosine hydroxylase) and renal afferent nerves (calcitonin gene-related peptide), as well as renal norepinephrine levels, were normal 11 months post-denervation. Findings at 5.5 months post-denervation were similar (n=5).

**Conclusions:** Catheter-based renal denervation effectively ablated the renal afferent and efferent nerves in normotensive sheep. By 11 months after denervation the functional afferent and efferent responses to electrical stimulation were normal. Reinnervation at 11 months post-denervation was supported by normal anatomical distribution of afferent and efferent renal nerves. In view of this evidence the mechanisms underlying the prolonged hypotensive effect of catheter-based renal denervation in human resistant hypertension need to be reassessed.

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**09: ANTI-HYPTERTENSIVE TREATMENT AND CEREBRAL BLOOD FLOW IN HUMAN HYPERTENSION**

**Hart E, Ratcliffe LE, Burchell AE, Nightingale AK, Wise R, Paton JFR**

aSchool of Physiology and Pharmacology, University of Bristol, Bristol, UK; bSchool of Clinical Sciences, University of Bristol, Bristol, UK; cCardiology, University Hospitals Bristol NHS Trust and Foundation, Bristol, UK; dCardiff University Brain Research Imaging Centre, Cardiff University, UK

**Background:** The onset of high blood pressure may be due to poor cerebral circulation. Additionally, hypertension...
is related to the development of dementia. Previous work suggests that angiotensin converting enzyme inhibitors (ACEi) improve cerebral blood flow and prevent pathological remodelling of the cerebral vessels when administered to young, hypertensive animals.

**Aim:** To measure whether cerebral blood flow was higher in hypertensive humans taking ACEi

**Methods:** The study involved comparison of hypertensive subjects being treated with ACEi (n=14 [9 being men], age; 58±8 years) and hypertensive subjects taking other anti-hypertensive medications (n=12 [7 being men], age 57±6 years), comprising 6 on calcium channel blockers and 6 on angiotensin receptor blockers) and normotensive (NT) participants (n=13, 54±6 years). Blood flow in the left and right internal carotid and vertebral arteries was measured using magnetic resonance phase contrast imaging. Total cerebral blood flow was the sum of the mean blood flow in all vessels. Total cerebral vascular resistance (CVR) was calculated as mean arterial pressure/total flow during the MR scan.

**Results:** Average 24 hour ambulatory day-time systolic and diastolic blood pressures in both treated groups were comparable (139±4/91±4 mmHg vs. 141±3/92±3 mmHg) and were higher than systolic pressure in the NT subjects (117±2 mmHg; P<0.05). The ACEi group had a total cerebral blood flow similar to NT (49±2 vs. 50±2 mL/100 mL/min), whereas the non ACEi group had a lower cerebral blood flow vs. NT (42±2 mL/100/mL/min; P<0.05). Importantly, CVR was increased in the non ACEi group vs. the NT group (1.6±0.1 vs. 2.4±0.2 mmHg•min/mL; P<0.05).

**Conclusion:** The present data provide preliminary evidence that ACEi might help to prevent a reduction in cerebral blood flow in hypertensive humans, potentially by preventing a rise in CVR.

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**10: BARORECEPTOR FUNCTION IS PRESERVED FOLLOWING FIELD STIMULATION OF CAROTID BARORECEPTORS IN NORMOTENSIVE AND HYPERTENSIVE RATS**

Kouchaki Z1, Butlin M1, Georgakopoulos D1, Avolio AP1

1Australian School of Advanced Medicine, Macquarie University, Sydney, New South Wales, Australia; 2CVRx Inc., Minneapolis, Minnesota, USA

**Background:** Field stimulation of the carotid baroreceptors has been successfully used to induce long-term reduction in blood pressure. However, whether baroreceptor stimulation may affect the short-term blood pressure regulation function of the baroreceptors in normotensive and hypertensive conditions is not well established.

**Aim:** To determine the effect of field stimulation of carotid baroreceptors on blood pressure in normotensive and hypertensive rats.

**Methods:** Male, Wistar Kyoto (WKY, n=7) and spontaneously hypertensive rats (SHR, n=7) (15–19 weeks old) were anaesthetized (urethane, 1.3 g/kg) and unilaterally vagotomized. Thoracic and aortic pressure was measured by an intravascular dual catheter tip pressure sensor (Science, 1.6F). Vessel stiffness was quantified by the pulse wave velocity (PWV) between the two pressure sensors. The left carotid artery was exposed and electrical field stimulation was applied to baroreceptors in the proximity of the carotid bifurcation (stimulation frequency: 100 Hz, pulse width: 0.53 ms, signal amplitude: 3-5 V). A bolus of phenylephrine (1.5 µg) was delivered during baseline (no stimulation) and during carotid baroreceptor stimulation to characterize baroreceptor function. Baroreceptor gain was computed as the absolute change in heart rate (HR) with respect to change in mean blood pressure (MAP).

**Results:** Stimulation caused a significant reduction in HR and MAP in both WKY (P=0.034 for HR, P=0.02 for MAP) and SHR (P=0.0007 for HR, P=0.002 for MAP), indicative of sympathetic inhibition. Field stimulation of the carotid baroreceptors did not affect the baroreceptor gain in either group. WKY: gain at baseline (no stimulation), 0.7±0.19 bpm/mmHg; gain during stimulation, 0.6±0.12 bpm/mmHg. SHR: gain at baseline (no stimulation), 0.36±0.10 bpm/mmHg; gain during stimulation, 0.44±0.12 bpm/mmHg. There was a non-significant trend for reduction in gain in SHR compared to WKY in both baseline and stimulation conditions. PVW did not change significantly with stimulation in both WKY and SHR.

**Conclusions:** Baroreceptor function was preserved during field stimulation of carotid baroreceptors in both WKY and SHR. This provides support for the use of field stimulation of baroreceptors as a means of blood pressure lowering therapy, whereby the acute and transient control of blood pressure in daily life is maintained.

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**11: ROLE OF THE RENAL NERVES IN A CONSCIOUS RABBIT MODEL OF CHRONIC KIDNEY DISEASE**

Head GA1, Burke SL1, van Rensch LM1, Sata Y3, Lambert GW1, Denton KM1, Schlaich MP3

1Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia; 2Department of Physiology, Monash University, Melbourne, Victoria, Australia

**Background:** Chronic kidney disease (CKD) contributes substantially to the global burden of cardiovascular disease. Nearly 1 in 3 Australians is at risk of developing CKD which is associated with activation of the sympathetic nervous system and elevated blood pressure (BP). The renal nerves are likely to play a major role in CKD-induced hypertension since chronic renal injury can stimulate afferent sensory nerve fibres which directly modulate central neuronal circuits and thus sympathetic outflow. We investigated a novel conscious rabbit model of chronic impaired renal function in which qualitative comparisons can be made of sympathetic activity. We determined the role of the renal nerves in the hypertension and altered autonomic reflex function which characterize the 5/6 renal nephrectomy model of CKD.

**Aim:** To determine whether renal nerves play a role in maintaining CKD-induced hypertension.

**Methods:** Chronic renal failure was induced by lesioning 5/6th of the glomerular layer of the renal cortex in one kidney and, after 2 weeks recovery, removing the contralateral kidney. We examined the role of the renal nerves...
by denervating the kidneys. Blood parameters, BP and renal sympathetic nerve activity (RSNA) and responses to stress (airjet) and hypoxia (10% O₂), baroreflexes and noradrenaline spillover were examined after 3 weeks of CKD.

**Results:** Plasma creatinine and urea were 44% and 38% higher in CKD than control rabbits (P<0.001). BP and RSNA were 8% and 33% higher (P<0.001), but total noradrenaline spillover was 32% lower in CKD rabbits (P<0.05). Responses to hypoxia were attenuated by CKD and RSNA baroreflexes were shifted to the higher BP level. Renal denervation reduced RSNA in both CKD and control rabbits (30%), but only lowered BP in controls. Noradrenaline spillover was unaltered by denervation but RSNA baroreflexes in both groups were shifted downwards so that both maximum and minimum RSNA were reduced. The hypertension to airjet was enhanced in both groups after denervation but the hypertension to hypoxia was attenuated in the control animals only. Blood parameters were not altered by renal denervation.

**Conclusion:** Our results show that while RSNA is elevated in CKD, there is sympathoinhibition in other beds. Renal denervation did not alter the renal impairment or the hypertension suggesting that the renal nerves play a minor role in maintaining CKD-induced hypertension in this model of CKD.

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**12: G PROTEIN-COUPLED ESTROGEN RECEPTOR SIGNALING IMPROVES STROKE OUTCOME IN FEMALE MICE**

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**Background:** Estrogen has been assumed to provide neuroprotection following stroke entirely via classical estrogen receptors. Interestingly, there is recent evidence that activation of a novel G protein-coupled estrogen receptor (GPER) with the selective ligand G-1 can improve stroke outcome in ovariectomized mice. However, it remains to be determined if the neuroprotection provided by endogenous estrogen occurs via GPER signaling.

**Aims:** To test if the selective GPER antagonist G-15 worsens stroke outcome and to examine whether tamoxifen, a clinically approved GPER agonist, provides neuroprotection post-stroke.

**Methods:** To address the first aim, intact female C57Bl6 mice were treated i.p. with G-15 (300 μg/kg, n=7) or vehicle (dimethyl sulfoxide, n=8) 1 h prior to 0.5 h middle cerebral artery occlusion (MCAO). To address the second aim, ovariectomized mice were treated i.p. with tamoxifen (10 μg/kg, n=8), vehicle (dimethyl sulfoxide, n=7), or a combination of tamoxifen and G-15 (n=5) 1 h prior to MCAO. In addition, T cell or neutrophil infiltration into the ischemic hemisphere was examined using CD3 or myeloperoxidase immunohistochemistry, respectively.

**Results:** After 24 h, intact female mice treated with G-15 showed worsened functional outcomes and increased infarct damage compared with vehicle. Furthermore, immunohistochemistry showed a significant increase in neutrophils, but not T lymphocytes in the ischemic hemisphere of G-15-treated mice. Tamoxifen-treated mice had significantly improved functional outcomes and ~60% smaller infarct volume (P<0.05) compared to vehicle. The neuroprotective effects of tamoxifen were blocked in the presence of G-15. Immunohistochemistry revealed that tamoxifen limited the infiltration of T lymphocytes and neutrophils within the ischemic hemisphere.

**Conclusions:** These results suggest that in females, GPER activation contributes to estrogen-mediated neuroprotection following stroke, and that tamoxifen can improve stroke outcome following surgical menopause.

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**13: CONTRIBUTION OF THE AREA POSTREMA TO THE INCREASED CARDIAC SYMPATHETIC NERVE ACTIVITY IN OVINE HEART FAILURE**

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**Background:** Heart Failure (HF) is associated with an increase in cardiac sympathetic nerve activity (CSNA), which is directly linked to mortality in HF patients. The mechanisms responsible for the elevated CSNA remain unclear. Previous studies indicate that the area postrema (AP), a circumventricular organ in the brainstem, plays a role in the control of sympathetic nerve activity. We hypothesized that the elevated CSNA in HF is mediated by the AP and lesioning this region would reduce the increased CSNA in sheep with HF.

**Aims:** To determine the effect of sham lesion or lesion of the AP on CSNA and hemodynamics in conscious sheep with HF.

**Methods:** Studies were conducted in 2 groups of sheep with pacing-induced HF: sham (n=6) and AP lesion (n=6) sheep. Mean arterial blood pressure (MAP), heart rate (HR) and CSNA were recorded simultaneously in conscious sheep, at least 4 days after surgery.

**Results:** Heart failure was associated with a significant decrease in ejection fraction (from 74±2 % to 38±1 %; P<0.001), which was similar in both groups. There was a significant reduction in CSNA burst incidence in the AP lesion group compared with the sham group (45±10 and 89±3 bursts/100 heartbeats, respectively; P<0.01). These data suggest that the AP plays a role in setting the detrimental high levels of CSNA in HF.
Background: Water is essential for life. The mechanisms regulating water homeostasis have been studied extensively. However, the impact of differences in patterns of water intake on cardiovascular health has largely been ignored. Evidence links recurrent dehydration, caused by periodic water intake, and chronic kidney disease. It is our hypothesis that restricted access to water will promote cardiovascular and renal disease, particularly in at risk populations.

Aim: To evaluate the chronic effects of periodic drinking on kidney function, blood pressure and immune cell infiltration in rats with pre-existing hypertension.

Methods: Mean arterial pressure (MAP) was measured continuously in male spontaneously hypertensive rats (SHR) that were only allowed access to water for 2 hours per day versus control rats that were given unrestricted access to water over a 4 week period. Urine osmolality was assessed via metabolic cage studies and glomerular filtration rate (GFR) was measured transcutaneously using FITC-labeled sinistrin at baseline and at the end of the 4 week treatment period. Finally, flow cytometry was performed to determine renal immune cell infiltration and cytokine production.

Results: Basal MAP and renal function were similar between the treatment groups. Across the 4 week treatment period, MAP was significantly higher in the water-restricted SHR than the control SHR (13 mmHg; P<0.05). Furthermore, by the study end GFR had decreased by 29 ± 6 % from baseline in the water-restricted SHR (P<0.001). In comparison, GFR was similar at baseline and study end in the control SHR. The increase in MAP and reduced renal function in the water-restricted versus control SHR was associated with higher urine osmolality (∼50%; P<0.05), which is indicative of increased plasma arginine vasopressin levels and dehydration. Although renal immune cell infiltration was similar between the groups, we observed a phenotypic shift towards the pro-inflammatory Th1 phenotype in kidney T cell infiltrate from water-restricted SHR.

Conclusions: Recurrent dehydration associated with chronic periodic drinking exacerbates hypertension, renal dysfunction and inflammation in male SHR. This highlights the importance of regular daily water intake for the maintenance of kidney health, particularly in populations with existing cardiovascular and renal disease.

15: TH2-PROMOTING CYTOKINE TREATMENT LIMITS BRAIN INJURY AFTER CEREBRAL ISCHEMIA IN TH1-DOMINANT MICE


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Background: Stroke is the second leading cause of death and leading cause of permanent disability worldwide. Current treatment with recombinant tissue plasminogen activator can only be administered within 4.5 h of stroke onset, benefiting less than 10% of all stroke patients. Following ischemic stroke, inflammation occurs in the brain and is a major contributor to secondary injury and tissue infarction. Previous evidence indicates that T-helper type-1 (Th1) immunity is associated with a worse outcome in Th1-dominant versus Th2-dominant mouse strains after stroke. It is, however, unknown whether brain injury and functional deficits can be limited by acute therapy to promote Th2-type immunity.

Aims: To test if Th2-promoting cytokines are able to switch the immune response in Th1-dominant C57BL/6 mice to a Th2-dominant phenotype, leading to reduced brain infarct, less inflammation and improved functional outcome after stroke.

Methods: Male mice were treated with vehicle, IL-4 or IL-33 (1% bovine serum albumin, 5 μg or 2 μg, respectively, i.p.) 24 h before and 1 h after cerebral ischemia. Mice were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) i.p. prior to middle cerebral artery occlusion (1 h). Neurological and hanging wire assessments were performed 24 h after stroke. Brains were removed and frozen brain sections (30 μm) were stained with thionin for infarct analysis. Brain infiltrating leukocytes were stained and quantified using flow cytometry. Antibiotics (ampicillin and gentamycin, 300 mg/kg and 12 mg/kg, respectively, s.c.) were administered to 6 mice n = 6 in combination with IL-33.

Results: Brain infarction was reduced by ∼35% by IL-33 or IL-4 treatment, as compared to vehicle (26.1±3.1 mm³, 29.1±3.7 mm³ and 43.8±4.6 mm³, respectively; n=13–16; both P<0.05). Mortality and neurological deficit were, however, exacerbated by IL-33 and IL-4 (n >18; both P<0.01). Flow cytometric analysis indicated that IL-33 reduced Ly6C+ monocyte numbers in the ischemic brain, as compared to vehicle (227±117 versus 782±279 cells, respectively; n=6–9; P<0.05). Ongoing studies suggest that combined IL-33 and antibiotic therapy improves functional recovery compared with IL-33 alone.

Conclusions: The present data indicate that acute administration of Th2-promoting cytokine limits brain injury but exacerbates functional deficit after stroke, possibly due to increased bacterial infections. Post-stroke cytokine therapy may be feasible, used together with antibiotics.
Therapeutic targeting of platelet cell death pathways may help reduce remote organ injury in critically ill patients. Studies demonstrate a new microvascular thrombosis mechanism that is triggered by membrane fragments from dying platelets.

18: THE SHEAR EXCITEMENT OF PLATELETS

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The platelet collagen receptor, glycoprotein (GP)VI of the immunoglobulin (Ig) superfamily, plays a key role in initiating platelet adhesion, activation and thrombus formation following vascular injury or alterations to blood rheology shear stress conditions. GPVI forms a functional complex with the Immune receptor Tyrosine-based Activation Motif (ITAM)-bearing Fc receptor y-chain (FcRy), required for surface expression of GPVI/FcRy. Surface expression of GPVI is also regulated by irreversible ectodomain shedding, mediated by the sheddase ADAM10 (of the ADAM family of a disintegrin and metalloproteinase-like proteins).
metalloproteinase family). This is based on studies showing ADAM10 (but not ADAM17) cleaves synthetic peptides based on the cleavage site in human GPVI, and the ADAM10-selective hydroxamate inhibitor GI254023 preferentially blocks shedding of GPVI. Western blot analysis reveals that essentially all of the GPVI on healthy circulating platelets is intact. However shedding is induced in vitro by exposure to elevated shear stress, GPVI ligands, coagulation or antiplatelet antibodies acting via the low-affinity IgG receptor, FcγRIIa. Soluble GPVI (sGPVI) is also elevated in plasma from patients with atherothrombotic disease or coagulopathy, immune or non-immune thrombocytopenia, and other diseases.

While GPVI shedding is rapidly induced (within seconds to minutes) when human platelets are treated with triggers of shedding, how ADAM10 activity and GPVI expression is regulated during thrombus formation is unknown. To address this question, we have used antibodies and fluorescence resonance energy transfer (FRET) substrates (GPVI-Cy3) to monitor sheddase activity on a forming thrombus. GPVI-Cy3 involves a fluorophore and quencher linked by a short peptide with sequence corresponding to the ADAM10 cleavage site in GPVI. GPVI-Cy3 is cleaved by recombinant ADAM10 (rADAM10) but not rADAM17, and when washed human platelets either treated with agents known to upregulate ADAM activity on other cells, or briefly exposed to pathological shear rates using a cone-plate viscometer. Using GPVI-Cy3 and DyLight-1G5 Fab fragment against GPVI, and confocal imaging of thrombi formed on a collagen surface under flow (input wall shear, 1800 s⁻¹), current studies are attempting to unravel mechanisms affecting thrombus growth and stability. Understanding of ADAM10 activity towards GPVI and other substrates of this ubiquitous enzyme may be relevant to bleeding or thrombotic propensity as well as vascular pathology beyond platelets and thrombosis.

19: DIABETES INCREASES RETICULATED PLATELETS DUE TO ENHANCED PROLIFERATION AND EXPANSION OF BONE MARROW MEGAKARYOCYTE PROGENITORS

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Diabetes (type 1 & 2) is a major risk factor for cardiovascular disease (CVD) which represents the major cause of mortality in this group. Patients with diabetes present with reticulated thrombocytosis (increased immature platelets), which lower the efficacy of current anti-platelet therapies. We suggest that the reticulated thrombocytosis observed in people with diabetes plays an important role in atherogenesis. Thus, we investigated the mechanisms contributing to increased reticulated platelets in diabetes. Streptozotocin (STZ) induced diabetic mice had significantly higher levels of reticulated platelets. This was paralleled by an increase in the population of megakaryocyte progenitors (MkPs) and megakaryocytes in the bone marrow (BM). Diabetes caused an increase in circulating thrombopoietin (TPO) without altering the expression of the TPO receptor (c-MPL) on any of the BM progenitor cells or circulating platelets. TPO expression is generally upregulated by Kupffer cell derived interleukin-6 (IL-6). We found more Kupffier cells in the liver of diabetic mice which expressed higher levels of cell surface RAGE (Receptor for Advanced Glycated End-products) or circulating platelets. TPO expression is generally upregulated by Kupffer cell derived interleukin-6 (IL-6). We found more Kupffier cells in the liver of diabetic mice which expressed higher levels of cell surface RAGE (Receptor for Advanced Glycated End-products) and produced more IL-6. Depletion of Kupffer cells using clodronate liposomes normalized the levels of total and reticulated platelets along with MkPs in the BM. Rage-/− BM transplantation (BMT) into mice that were then rendered diabetic were protected from diabetes-induced thrombocytosis. We noted that Kupffer cells from diabetic Rage-/− BMT mice failed to express IL-6 and plasma TPO levels remained similar to the non-diabetic mice. Rage-/− BMT mice also had normal levels of MkPs in the BM. Thus, we have identified a role for RAGE in the liver Kupffer cells of diabetic mice that triggers IL-6 expression, which in-turn promotes TPO production. TPO then enhances proliferation and expansion of MkPs in the BM, resulting in an increase in the abundance of reticulated platelets which could contribute to accelerated atherogenesis in diabetes.

20: MOLECULAR ULTRASOUND IMAGING USING PLATELET-TARGETED MICROBUBBLES: DIAGNOSIS, MONITORING AND EFFICACY TESTING OF THROMBOLYTIC DRUGS

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Introduction: Molecular ultrasound imaging offers a non-invasive technology widely available for rapid clinical diagnosis. We tested whether microbubbles (MBs), which are selectively targeted to activated platelets, provide a high-resolution, real-time imaging of thrombosis and monitoring of thrombolysis. We used this approach to evaluate a platelet targeted urokinase plasminogen activator (targ-scuPA) that is hypothesized to offer anti-thrombolytic potency without bleeding complications.

Methods and Results: MBs were conjugated to a single-chain antibody specific for an epitope called Ligand Induced Binding Site on activated GPIIb/IIIa (LIBS-MB). LIBS-MBs strongly adhered to immobilized activated platelets and micro-thrombi under flow. Carotid artery thrombi in mice, induced by ferric chloride, were assessed with ultrasound before and after MB injection. Analysis of the thrombus area demonstrated a significant increase in decibel after LIBS-MB but not after MB injection (p<0.01). After thrombolysis with 500U/g BW of commercial urokinase (commUPA), LIBS-MB ultrasound imaging allows monitoring of the reduction in thrombus size (p<0.001). Similar results were obtained when comparing the size to grayscale intensity reduction. In addition, 75U/g BW of targ-scuPA is sufficient for thrombolysis, whereas 75U/g BW of commUPA or non-targ-scuPA are not (p<0.01). 500U/g BW of commUPA, the concentration required to match the effectiveness of 75U/g BW of targ-scuPA, resulted in prolonged tail bleeding time, whereas no increase in bleeding was observed when the equally effective but lower dose of 75U/g BW scuPA (p<0.001).

Conclusion: We are able to demonstrate that our targeted MB specifically bind to activated platelets enabling real-
time molecular ultrasound imaging of thrombosis and monitoring of success or failure of thrombolysis in vivo. In an exemplary application a highly promising clot-targeted thrombolytic drug was shown to provide effective thrombolytic potential without compromising haemostasis.

China-Aus Vascular Biology / ASCEPT

21: MACROPHAGES AS MEDIATORS OF VESSEL REMODELLING IN HYPERTENSION

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It has long been known that hypertension is associated with the accumulation of macrophages in key blood pressure-regulating organs such as the blood vessels and kidneys. However, little is known about the activation state of such cells (M1/M2) or the role they play in the renal and vascular pathologies associated with the condition. Our recent work suggests that the macrophages that enter the aortic wall during hypertension become polarized towards an M2 phenotype.

Through their ability to release pro-fibrotic factors such as transforming growth factor-β and fibronectin, as well as trophic factors such as insulin-like growth factor and fibroblast growth factor, M2 macrophages are predicted to be major contributors to the vascular remodelling that ultimately contributes to vessel stiffening and its sequelae of elevated systolic blood pressure, left ventricular hypertrophy and end organ damage. Thus, we propose that strategies that inhibit the accumulation and subsequent M2 polarization of macrophages in the vessel wall may hold promise as future treatments for hypertension.

22: MATRIX METABOLISM AND CARDIOVASCULAR DISEASE

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Cardiovascular remodeling, defined as any enduring change in the size and/or composition of an adult heart or blood vessel, underlines the pathogenesis of major cardiovascular diseases. Cumulative studies have demonstrated that extracellular matrix (ECM) turnover play a critical role during cardiovascular remodeling. Recently, we find a 524-kDa matricellular glycoprotein, cartilage oligomeric matrix protein (COMP), pivotal for maintaining the homeostasis of vascular smooth muscle cells (VSMCs) in vessels and heart. COMP is essential for maintaining a VSMC contractile phenotype and the protective effects of COMP are mainly mediated through interaction with α7 integrin. Additionally, COMP binds directly to BMP-2 (bone morphogenetic proteins), inhibits BMP-2 receptor binding and osteochondrogenic transition of VSMCs, and ultimately prevents vascular calcification. Ablation or degradation of COMP facilitates VSMCs migration, enhances intima hyperplasia with injury, and predisposes vessels to calcification. Moreover, COMP−/− mice develops dilated cardiomyopathy (DCM) spontaneously at young age, with impaired cardiac function. COMP directly binds to the extracellular β-tail domain of integrin β1, prevents integrin β1 ubiquitination/degradation, and maintains the cardiac homeostasis. Manipulation of COMP level may shed light on the prevention and treatment of a variety of cardiovascular diseases.

23: SENESCENT ENDOTHELIAL CELLS SHOW AN UNIQUE ANTI-INFLAMMATORY PHENOTYPE

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Endothelial cells are one of the key cells in the regulation of acute and chronic inflammation, controlling the magnitude and duration of the response. Upon activation by inflammatory cytokines, EC not only induce a spectrum of pro-inflammatory activators, but there also occurs induction of negative regulators in a self-regulatory loop to limit or inhibit the activation status. Inflammation and senescence have been linked at both the clinical and molecular levels. In general, senescent cells have been described as pro-inflammatory based on their senescence associated secretory phenotype (SASP). However, endothelial cells when induced to become senescent by the chief signals associated with ageing; oxidative stress, disturbed flow and hypoxia display both anti-inflammatory and pro-inflammatory senescent phenotypes. Further, the anti-inflammatory phenotype is also seen in senescence induced by overexpression of ARHGAP18 or SENEX. The anti-inflammatory phenotype of endothelial cells is mediated through caveolae.

We hypothesize that the anti-inflammatory population of senescent endothelial cells is in in keeping with the concept of the endothelium being important to induce and resolve inflammatory responses. Thus the senescent endothelium may have a unique protective role, to inhibit uncontrolled proliferation and to limit the local inflammatory response.
24: ATHEROSCLEROSIS IN DIABETES: METABOLIC KARMA?

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Diabetes continues to cast a long shadow over the lives of many people. It is now clear that even transient hyper- or hypoglycemia or increased glycemic variability around healthy mean glucose levels can have long-lasting and long-term effects on the development and progression of diabetic complications, including cardiovascular disease. Even after glycemic control has been achieved and maintained for many years, it appears hard to undo the changes that are instilled, including epigenetic programming, compositional changes, post-translational modifications, or simply lead time toward an inevitable fate. This phenomenon has become known as “metabolic memory” or the “legacy effect,” but it may be better characterized as “metabolic karma,” in which the intent and actions of an individual (with respect to metabolic control) influence the future health of that individual. This “bad karma” has been used to explain many clinical observations surrounding diabetes and its management, including the lack of benefits in many short- and intermediate-term trials, and the potential utility of early intensive glycemic control. Further understanding the molecular basis of a metabolic legacy in diabetes will certainly provide new targets for intervention.

25: MECHANISMS OF MYELOPOIESIS IN OBESITY: ROLE OF ADIPOSE TISSUE MACROPHAGES

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Obesity is associated with infiltration of adipose tissue with inflammatory macrophages (ATMs), contributing to insulin resistance and diabetes. However, relatively little is known regarding the source of ATMs. We discovered that murine models of obesity exhibit prominent monocytopoiesis and neutrophilia due to proliferation and expansion of myeloid progenitor populations in the bone marrow (BM). Fat transplantation and weight loss studies (both in mice and humans) revealed that source of ligands that promote myelopoiesis was adipose tissue (AT). Mechanistic studies confirmed that myelopoiesis was driven by AT-derived damage associated molecular patterns (DAMPs) such as S100A8/A9, acting on TLR4 and activating the Nlpr3 inflammasome on ATMs. These events led to the synthesis and release of IL-1β, which in turn interacted with IL-1R on myeloid progenitor cells in the BM to promote myelopoiesis. These studies uncover a positive feedback loop between ATMs and BM myeloid progenitors to promote myelopoiesis and insulin resistance in obesity.

26 FIBROUS CAP THICKNESS OF NON-CULPRIT PLAQUES IN DIABETIC AND NON-DIABETIC PATIENTS IN RESPONSE TO LDL-C LOWERING THERAPY: INSIGHTS FROM FREQUENCY-DOMAIN OPTICAL COHERENCE TOMOGRAPHY ANALYSIS

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Background and Aims: Intensive lowering LDL-C with statins reduces cardiovascular events and slows plaque progression. Despite these beneficial effects, diabetic patients still have worse clinical outcomes compared with non-diabetics. The mechanisms that underlie adverse cardiovascular outcomes in diabetics have not been well characterized. Frequency-domain optical coherence tomography (FD-OCT) enables evaluation of plaque microstructures associated with plaque instability. We investigated whether plaque microstructures in diabetics exhibited different responses to LDL-C lowering therapy.

Methods: 293 non-culprit lipid plaques in 170 non-diabetic and 110 diabetic CAD patients treated with statin were evaluated by FD-OCT imaging in the entire of target vessel requiring percutaneous coronary intervention. These plaques were stratified into 3 groups in non-diabetic and diabetics according to attained LDL-C level under statin therapy (<1.8, 1.8-2.6 and <2.6 mmol/L). Clinical characteristics and FD-OCT derived fibrous cap thickness were compared.

Results: 47.0% of non-diabetics and 50% of diabetics were treated with high-dose statins (p=0.70). There were no significant differences in the prevalence of non-diabetics and diabetics who achieved LDL-C <2.6 and 1.8 mmol/L, respectively (63.1% vs. 71.9%, p=0.25; 29.8 vs. 19.8%, p=0.14). In non-diabetic subjects, patients with LDL-C <1.8 mmol/L were more likely to be younger (p=0.04), male (p=0.0001) and have a lower level of fasting blood glucose (p=0.04). FD-OCT analysis demonstrated that achieving a lower LDL-C level associated with thicker fibrous caps (150±93 vs. 112±57 vs. 95±39, p<0.01). Diabetic patients with LDL-C <1.8 mmol/L were likely to be older (p=0.0001), male (p=0.05) and have lower levels of BMI (p=0.01) and fasting blood glucose (p=0.01). In contrast to non-diabetics, fibrous cap thickness was comparable regardless of achieving lower LDL-C level (136±78 vs. 119±43 vs. 104±72 um, p=0.33 for trend). Multivariate analysis indicated that LDL-C level <1.8 mmol/L was a significant predictor of thicker fibrous cap in non-diabetics (p=0.02) but not diabetics (p=0.14).

Conclusions: The beneficial effects of LDL-C lowering therapy with statin was observed in non-diabetics but not diabetics. The attenuated efficacy of lowering LDL-C level may suggest the need for additional therapeutic approaches in diabetic patients with stable CAD.
Background: Evidence suggests that pancreatic lipid accumulation causes β-cell dysfunction. Mice with β-cell specific ABCA1 deletion and global knockout of ABCG1 have increased β-cell sterol levels, impaired glucose tolerance, raised fasting plasma glucose levels and impaired insulin secretion. However, as mice with global deletion of ABCG1 also have low adipose tissue mass and do not become glucose intolerant or insulin resistant when challenged with a high fat diet, that study did not provide an insight into the specific impact of β-cell dysfunction on glucose metabolism.

Aim: Investigate the effects of isolated β-cell-specific deletion of ABCA1 and ABCG1 on glucose metabolism in mice.

Methods: β-cell specific knockout mice were generated by crossing Ins2-cre and Abca1fl/flAbcg1fl/fl mice. Animals were maintained on a chow diet from weaning until 16 weeks of age. Body weight and food intake were monitored weekly. At 16 weeks of age, mice were subjected to glucose (2 g/kg) and insulin (1 U/kg) tolerance tests. Blood glucose and insulin levels were measured with a glucometer and by RIA, respectively. Plasma MCP-1 and IL-6 levels were measured by ELISA. Fat and muscle mass were assessed by MRI. Insulin levels in knockout mice were restored to normal by subcutaneously inserting osmotic pumps filled with Humulin R (31 U/ml).

Results: Islet ABCA1 and ABCG1 expression was reduced by 76% and 70%, respectively, in knockout mice compared to control but comparable in all other tissues. Control and knockout animals had comparable islet mass and insulin content, but knockout mice were markedly glucose intolerant (AUC 2649±230 vs 1539±189) and had reduced fasting insulin levels (0.67±0.18 vs 1.11±0.17 ng/ml). Insulin levels increased 1.5 fold in response to a glucose challenge in control animals, while no increase was observed in knockout animals. Both knockout and control animals were insulin sensitive. Despite similar weight and food intake, knockout mice had a 28% increase in adiposity and a 10% reduction in muscle mass and plasma IL-6 and MCP-1 levels were increased 3.5- and 5-fold, respectively, relative to the control animals. Adipose accumulation was attenuated when insulin levels in knockout mice were returned to normal using osmotic pumps between 12 and 16 weeks of age (0.88±1.07% vs 4.03±1.09% increase in adiposity for insulin and PBS treated animals, respectively).

Conclusion: β-cell specific deletion of ABCA1 and ABCG1 in mice reduces secretion which in turn impairs glucose metabolism, alters body composition and increases systemic inflammation.

Introduction: Disordered neovascularisation, impaired wound healing and aberrant vascular endothelial growth factor (VEGF) production and signaling, are conspicuous features of diabetic vascular diseases. Recent studies show infusions of apolipoprotein (apo) A-I, the main protein component of high-density lipoproteins (HDL), augment ischaemia-driven neovascularisation. We now aim to determine if HDL can rescue diabetes-impaired angiogenesis.

Methods and Results: In vitro, in human coronary artery endothelial cells (HCAECs), pre-incubation with reconstituted HDL (rHDL, apoA-I + phosphatidylcholine) rescued hyperglycaemic (25mM glucose) impairment of matrigel tubulogenesis (81%) and VEGF protein (76%), and promoted the phosphorylation of VEGF receptor 2 (pVEGFR2) protein (91%), compared to PBS controls, p<0.05. Cells pre-incubated with rHDL also had increased protein levels of phosphorylated eNOS (94%) and Akt (92%), two key pathways in angiogenesis, and elevated mRNA levels of Siah1 and Siah2 (2.4- and 2.2-fold increase respectively), which are involved in the stabilisation of HIF-1α, p<0.05 for all. Moreover, lentiviral shRNA knockdown of scavenger receptor-BI (SR-BI) in HCAECs abrogated rHDL rescue of hyperglycaemia-impaired tubule formation and VEGF protein levels. In vivo, diabetes was induced in wild type and SR-BI knockout (SR-BI/-) mice using streptozotocin. Diabetic mice had reduced blood flow recovery (53%) and neovessel formation (11%), compared to non-diabetic mice in the hindlimb ischaemia model, p<0.05. Intravenous infusions of rHDL completely restored diabetes-impaired blood flow and capillary density to non-diabetic levels, and augmented hindlimb mRNA levels of VEGF (53%), p<0.05. In a murine wound-healing model, diabetic mice exhibited delayed wound closure (36%), p<0.05. However, topical application of rHDL accelerated wound closure (41%) and wound angiogenesis (22%), and increased capillary density (130%) in diabetic mice, compared to PBS treated wounds, p<0.05. In SR-BI/- diabetic and non-diabetic mice, rHDL was unable to induce angiogenesis in the hindlimb ischaemia model or increase wound closure/angiogenesis.

Conclusion: rHDL rescues diabetes-impaired angiogenesis with restoration of wound healing and ischemia-induced angiogenesis to that of non-diabetic animals. Mechanistically, these actions appear to be mediated through the restoration of VEGF, VEGFR2 phosphorylation, HIF-1α stabilisation, eNOS and the SR-BI receptor. These findings have significant implications for the therapeutic modulation of diabetic vascular complications.
**Background:** Obesity is characterised by adipose tissue inflammation and increased secretion of pro-inflammatory adipokines, such as monocyte chemotactic protein-1 (MCP-1) and interleukin-6 (IL-6). A plausible mechanism for these effects is an increase in mitogen-activated protein kinase (MAPK)-mediated signalling pathways, including p38 (p38MAPK), c-Jun N-terminal kinase (JNK) and extracellular signal-related kinase (ERK). A well-known MAPK activator is toll-like receptor 4 (TLR4), which also triggers downstream activation of a key inflammatory regulator, nuclear factor-κB (NF-κB). Apolipoprotein A-I (apoA-I), the main lipoprotein of high density lipoproteins, attenuates TLR4 signal transduction pathways and NF-κB activation in endothelial cells. ApoA-I is also the primary acceptor of cholesterol that is exported from cells expressing the ATP-binding cholesterol transporter, ABCA1. The roles of apoA-I and ABCA1 in the inhibition of inflammation in adipocytes are unknown.

**Aim:** To determine if apoA-I inhibits inflammation and inflammatory signalling pathways in lipopolysaccharide (LPS)-stimulated 3T3-L1 adipocytes.

**Methods:** Mature 3T3-L1 adipocytes were pre-incubated for 16 h with or without apoA-I (1 mg/mL), then stimulated with LPS (100 ng/mL). MCP-1 and IL-6 secretion and synthesis were quantified by ELISA and qPCR, respectively. ERK1/2, p38MAPK, JNK1/2 phosphorylation and nuclear localisation of NF-κB p65 were quantified by western blotting. Cell surface expression of TLR4 was analysed by flow cytometry. ABCA1 expression was silenced by transfection with ABCA1 siRNA.

**Results:** Pre-incubation of 3T3-L1 cells with apoA-I reduced the LPS-mediated secretion of MCP-1 from 2666±216 to 983±65 pg/mg cell protein, while IL-6 levels decreased from 120±22 to 55±11 pg/mg cell protein. MCP-1 and IL-6 mRNA levels decreased by 43- and 5-fold, respectively (p<0.05 for all). These effects of apoA-I were attenuated in ABCA1siRNA-transfected cells. ApoA-I inhibited LPS-stimulated phosphorylation of ERK1/2, p38MAPK and JNK1/2 by 66.2±4%, 58.8±10.7% and 33.2±14%, respectively, while nuclear NF-κB p65 levels were decreased by 32.6±0.9% (p<0.05 for all). Pre-incubation of 3T3-L1 cells with apoA-I also decreased cell surface TLR4 protein levels by 26±3% (p<0.0001).

**Conclusion:** ApoA-I reduces TLR4 expression, MAPK signalling pathways and NF-κB activation in 3T3-L1 adipocytes. It also inhibits the synthesis and secretion of pro-inflammatory adipokines in 3T3-L1 adipocytes in an ABCA1-dependent manner.

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**30: RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF VITAMIN D SUPPLEMENTATION ON PERIPHERAL AND CENTRAL BLOOD PRESSURE, VISIT-TO-VISIT BLOOD PRESSURE VARIABILITY AND AORTIC STIFFNESS IN OLDER INDIVIDUALS**

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**Background:** Observational studies report inverse relationships of serum vitamin D with blood pressure (BP) indices and aortic stiffness. This suggests that vitamin D supplementation may have cardiovascular benefits, especially in people with low vitamin D, but there is limited intervention data to support this hypothesis.

**Aim:** To determine the effect of vitamin D supplementation on BP indices (including BP variability [BPV]) and aortic stiffness in people with vitamin D deficiency.

**Methods.** In a double-blind, placebo-controlled trial, 239 older individuals (aged 63±7 years; female 50%) with vitamin D deficiency (<60 but >12.5 nmol/L) were randomized to 12-months intervention (vitamin D3 50,000 U/month; n=118) or matching placebo (n=121). Brachial and central BP, as well as visit-to-visit BPV and aortic stiffness (carotid-femoral pulse wave velocity) were measured at baseline, 6 and 12 months.

**Results:** There was a significant increase in serum 25-hydroxy-vitamin D with intervention compared to placebo (45.1 [95% CI 40.2–49.9] vs. 8.5 [95% CI 3.8–13.2] nmol/L; P<0.001). However, intervention failed to produce any clinical or statistically significant changes to brachial systolic BP (–2.79 [95% CI –5.40 to –0.18] vs. –3.25 [–5.89 to –0.61] mmHg; P=0.8), central BP or BPV indices (all P>0.05), or aortic stiffness (–0.22 [95% CI –0.59 to 0.14] vs. 0.06 [–0.32 to 0.43] m/s; P=0.30).

**Conclusions:** Despite many observational studies suggesting that vitamin D supplementation could be useful therapy for lowering BP and aortic stiffness, 12-months intervention yielded no improvement in older people with vitamin D deficiency. These results do not support use of vitamin D supplementation to improve cardiovascular health in this population.

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**31: CARDIAC ACTIN-MYOSIN CROSS-BRIDGE DYSREGULATION OCCURS EARLY IN THE PATHOGENESIS OF TYPE 2 DIABETIC CARDIOMYOPATHY**

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**Background:** The prevalence of insulin resistance (IR) and type 2 diabetes (T2DM) are rapidly becoming a global epidemic. Individuals with IR and T2DM have a significantly increased risk of developing cardiovascular disease,
including diabetic cardiomyopathy. Diastolic dysfunction associated with diabetic cardiomyopathy is often attributed to increased collagen deposition, extracellular matrix accumulation and hypertrophy. Moreover, impaired diastolic function often occurs ahead of structural remodelling early in the pathogenesis of diabetic cardiomyopathy. Currently, the precise molecular mechanisms leading to the development of early contractile dysfunction in diabetic cardiomyopathy remain largely unclear. One possible contributor to early contractile dysfunction could be myofilament dysfunction. Using synchrotron radiation as a source for small angle x-ray scattering (SAXS), it is possible to evaluate real time cardiac actin-myosin cross-bridge (CB) dynamics in the *in situ* beating heart simultaneously with cardiac function by left pressure-volumetry. The Goto-Kakizaki (GK) rat is a model of progressive T2DM and by 10–12 weeks of age has established IR and mild hyperglycaemia.

**Aim:** To determine if IR resulted in impaired CB dynamics and cardiac dysfunction in young, insulin resistant GK rats.

**Methods:** Evaluation of CB dynamics was performed the *in situ* beating hearts of GK rats (10–12 weeks old, n=6) and age-matched Wistar control rats (n=7) at the beamline 40XU, SPring-8 Synchrotron, Japan. Under surgical anaesthesia, rats were thoracotomized and myocardial SAXS patterns were digitally recorded during baseline conditions and dobutamine stimulation.

**Results:** GK rats displayed cardiac hypertrophy and moderate hyperglycemia compared to Wistar rats. In the subendocardial and subepicardial layers of the heart, diastolic myosin head proximity to actin filaments was significantly reduced (P < 0.05 and P < 0.01, respectively) in comparison to Wistar rats, but was normalized during dobutamine infusion. As the calculated spacing between the myosin filaments did not differ between Wistar and GK rats, our data suggests diastolic myosin head extension is depressed in the deeper myocardial layers in the insulin resistant heart.

**Conclusions:** In the heart of young GK rats, CB dynamics in the subepicardium and subendocardium are slowed by increased separation of myosin heads and may be an early event that drives diastolic dysfunction in the diabetic heart.

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### 32: TREATMENT WITH THE MAS RECEPTOR AGONIST, AVE-0991, RESTORES THE NORMAL REGULATION OF ARTERIAL PRESSURE IN ACE2 DEFICIENT MICE

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**Background:** Pregnant women and gravid animal models of pregnancy are less sensitive to the pressor effects of angiotensin II (Ang II). Notably, angiotensin converting enzyme (ACE)-2 and Ang (1-7) plasma levels are increased during pregnancy, suggesting that the ACE2/Ang(1-7)/MasR pathway counters the pressor actions of Ang II during pregnancy.

**Aim:** To determine the contribution of ACE2 to the regulation of arterial pressure and immune cell infiltration during pregnancy.

**Methods:** Mean arterial pressure (MAP) was measured via telemetry in 14 week old wild-type (WT) and ACE2 knockout (ACE2-KO) mice receiving vehicle (saline, s.c.; n=10,7, respectively) or the MasR agonist, AVE-0991 (24 μg/kg/min, s.c; n=7,8, respectively) prior to and during pregnancy. In additional cohorts, renal excretory function was measured via collection of a 24h urine sample and renal angiotensin receptor (AT1aR, AT1bR, AT2R and MasR) mRNA levels were determined by real-time RT-PCR at baseline and on gestation day (Gd) 18. Circulating immune system activation and immune cell infiltration into the kidneys (baseline and Gd18) and placenta (Gd18) were examined using flow cytometry.

**Results:** Basal MAP was higher in ACE2-KO versus WT mice (100±1 and 94±1 mmHg, respectively; P=0.04). Treatment with AVE-0991 lowered basal MAP by 5±1 mmHg in ACE2-KO mice (P=0.007). In WT mice, MAP decreased during mid-gestation reaching a nadir at Gd9 (88±1 mmHg; P=0.007 versus basal) and returning to near pre-conception levels during late gestation. Whilst the normal gestational decrease in MAP was observed in ACE2-KO mice, MAP increased significantly during late gestation reaching a nadir at Gd9 (88±1 mmHg; AVE-0991 lowered basal MAP by 5±1 mmHg in ACE2-KO mice (P=0.007). In WT mice, MAP decreased during mid-gestation reaching a nadir at Gd9 (88±1 mmHg; P=0.007 versus basal) and returning to near pre-conception levels during late gestation. However, the increase in MAP was significantly reduced in ACE2-KO mice compared to WT mice (P<0.001). Moreover, the reduction in circulating T regulatory cells during pregnancy was greater in ACE2-KO than WT mice (P<0.001). At Gd18, gestational weight gain was lower in ACE2-KO mice compared to WT mice (13±1 versus 10±1, respectively; P=0.01). At birth, litter size was lower in ACE2-KO mice than WT mice (7±1 versus 5±1, respectively; P=0.04). AVE-0991 did not effect litter size or birth weight.

**Conclusions:** MasR stimulation restored the normal arterial pressure pattern during pregnancy in ACE2 deficient mice without effect on fetal wellbeing. The ACE2/Ang(1-7)/MasR pathway may represent a new therapeutic target for the treatment of pregnancy-induced hypertension.

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### 33: EFFECTS OF INSULIN REGULATED AMINOPEPTIDASE (IRAP) INHIBITION IN ANGIOTENSIN II-INDUCED HYPERTENSION

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**Background:** Insulin regulated aminopeptidase (IRAP), also known as the AT4 receptor (AT4R), is upregulated in animal models of vascular injury and atherosclerosis. Importantly, chronic treatment with the endogenous AT4R ligand, angiotensin (Ang) IV is both vaso- and athero-protective, with these actions hypothesized to be mediated via inhibition of IRAP’s catalytic activity.

**Aims:** To investigate whether IRAP deficiency or pharmacological inhibition of IRAP would prevent Ang II-induced abdominal aortic aneurysms (AAA) and vascular injury.

**Methods:** Adult male IRAP deficient [IRAP knockout (KO)] or WT (C57Bl/6J) mice aged between 4–6 months were infused with either saline or Ang II (4 weeks; 800 ng/kg/min). Other WT mice were co-treated with Ang II and...
either the synthetic IRAP inhibitor, HF419 (500 ng/kg/min) or vehicle for 4 weeks. At the end of treatment, incidence of AAA, vascular reactivity, aortic hypertrophy, fibrosis and inflammation was quantified.

**Results:** Ang II infusion significantly increased systolic blood pressure in WT mice, while IRAP deficiency or inhibition significantly reduced Ang II-induced hypertension. Strikingly, IRAP KO or HF419-treated WT mice exhibited reduced incidence of Ang II-evoked AAA, by ~65–80% (n=7–10, P<0.01). IRAP deletion or inhibition also attenuated Ang II-induced endothelial dysfunction, which was correlated with enhanced vascular phospho-eNOS, decreased NADPH oxidase (NOX)-2 and subsequent superoxide production (~50–90%; P<0.001). Ang II infusion increased IRAP and inflammatory markers such as NFkB, MCP-1 and ICAM-1, all of which were reduced with IRAP deficiency or inhibition. H&E staining further revealed that ablation of IRAP abolished Ang II-induced aortic hypertrophy which resulted in reduced adventitial thickening (~2-fold; P<0.05), a major site of inflammatory and fibrotic response. In addition, PSR-stained aortic sections revealed that Ang II-treated IRAP KO and HF419 group exhibited ~70% less collagen compared to WT controls (n=6; P<0.01), indicating reduced aortic fibrosis potentially due to prevention of Ang II-induced increase in myofibroblast expression (~50%; P<0.05).

**Conclusions:** Pharmacological inhibition of IRAP exerts antihypertensive, vasoprotective and anti-inflammatory effects, consistent with effects seen in IRAP deficient mice, thus highlighting the potential use of IRAP inhibitors as a future therapeutic treatment for cardiovascular disease.

**EASY CAREER RESEARCHER FINALISTS:**

### 34: RENAL SYMPATHETIC INNERVATION REGULATES BLOOD PRESSURE BY ACTIONS ON miR-181a AND RENIN

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**Background:** MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. We have shown previously that the microRNA miR-181a was able to bind to and regulate renin mRNA level in transfected kidney cells. This microRNA was down-regulated in Wistar rats and in the kidney of Schlager hypertensive model (BPH/2J), a neurogenic model of hypertension. Compared to their normotensive counterparts (BPN/3J), BPH/2J had a greater renal sympathetic innervation. Thus, we hypothesized that exaggerated sympathetic renal activity acting through miR-181a and renin was contributing to elevate blood pressure in BPH/2J.

**Aims:** To determine the effect of bilateral renal denervation (Rx) on miR-181a and renin mRNA levels in BPH/2J and BPN/3J mice.

**Methods:** Blood pressure was measured via pre-implanted radiotelemetry probes before and after 3 weeks of Rx (10% phenol) or sham surgery. Kidneys were collected following the final recording to measure noradrenaline content and extract RNA. Real-time PCR was used to measure renal levels of miR-181a and renin mRNA.

**Results:** Rx reduced blood pressure by 8.0±2.2 mmHg in BPH/2J mice over the 3 weeks of the experiment, but had no effect in BPN/3J. Following Rx, miR-181a levels were significantly higher in the BPH/2J (sham 0.66±0.02 vs Rx 0.72±0.02; P=0.020), while no difference was observed in BPN/3J (sham 0.73±0.03 vs Rx 0.73±0.03; P=0.46). Consistent with an effect of miR-181a on renin mRNA, renin mRNA was significantly lower in BPH/2J mice after Rx (sham 2.3±0.2 vs Rx 1.9±0.1; P=0.04), and no difference was observed in BPN/3J (sham 1.6±0.2 vs Rx 1.9±0.2; P=0.14). Rx normalized both miR-181a (0.72±0.02 BPH/2J vs 0.73±0.03 BPN/3J; P=0.84) and renin mRNA (1.9±0.1 BPH/2J vs 1.6±0.2 BPN/3J; P=0.11) in BPH/2J to levels comparable to the control strain.

**Conclusions:** Our study supports our hypothesis of involvement of renal sympathetic innervation in the regulation of blood pressure in hypertension through mechanisms involving miR-181a and renin.

### 35: THE CONTRIBUTION OF OREXIN TO THE NEUROGENIC HYPERTENSION IN BPH/2J MICE

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**Background:** Schlager BPH/2J mice are a genetic model of hypertension associated with an overactive sympathetic nervous system (SNS). Orexin is a neuropeptide which can regulate sympathetic activity, blood pressure and stress. Interestingly levels of orexin precursor mRNA are greater in the hypothalamus of BPH/2J mice compared with normotensive BPN/3J control mice, particularly during the dark period of the 24 hour light cycle when hypertension is at its greatest in these mice.

**Aim:** The aim of the present study was to determine whether enhanced orexigenic signaling contributes to hypertension in BPH/2J mice.

**Methods:** BPH/2J and BPN/3J mice (n=6–7) were pre-implanted with radiotelemetry probes to measure mean arterial pressure (MAP). The dual orexin receptor 1 and 2 antagonist, Almorexant (Actelion) was administered via intraperitoneal injection (i.p.) and gavage (p.o.). MAP was recorded for 6 hours and compared with baseline values 1 hour before treatment. Mid frequency (0.3–0.5 Hz) MAP power and the depression response to ganglion blockade were both used as indicators of SNS activity in vehicle and Almorexant (i.p. 100 mg/kg) treated mice (n=3–4).
Results: Administration of Almorexant at 100 mg/kg (i.p.) and 300 mg/kg (p.o.) during the dark period of the 24 hour light cycle caused sustained hypertensive responses in BPH/2J mice (~15.1±1.4 and ~10.4±1.1 mmHg, respectively), which were markedly greater than the effect of vehicle administration (~0.5±0.7 mmHg; *P<0.001). By contrast the responses to Almorexant in BPN/3J mice at all doses and routes were comparable with vehicle (*P=0.57). During the dark period Almorexant attenuated the depressor response to ganglion blockade in both BPH/2J and BPN/3J mice (*P=0.007). Almorexant treatment also reduced the mid frequency MAP power in BPH/2J mice (*P<0.001), but not BPN/3J mice (*P=0.65). During the light period, Almorexant (100 mg/kg, i.p.) did not reduce MAP from baseline in either strain, but a moderate pressor effect following vehicle injection was reduced following Almorexant treatment (13.3±1.7 mmHg vs 1.6±2.0 mmHg respectively; *P<0.001) in BPH/2J mice only.

Conclusion: The present results demonstrate that inhibition of orexin 1 and 2 receptors with Almorexant delivered either orally or i.p. can reduce BP and SNS activity in BPH/2J mice. These findings suggest that enhanced orexinergic signaling contributes to overactivation of the SNS and hypertension in BPH/2J mice, particularly during the dark period.

36: CLONIDINE RESTORES VASCULAR SENSITIVITY TO PHENYLEPHRINE AND ANGIOTENSIN II DURING HYPOTENSIVE SEPSIS IN CONSCIOUS SHEEP

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Background: Hypotension with vascular hyporesponsiveness to vasopressor therapy is both a significant and independent prognostic factor of mortality during septic shock. In sepsis, there is a large increase in sympathetic nerve activity resulting in high endogenous levels of catecholamines, which may cause a low vascular sensitivity to vasopressors. We hypothesized that sympathetic inhibition induced by the α₂-adrenoceptor agonist clonidine would reverse the vascular hyporeactivity to both endogenous and exogenously infused vasoconstrictors.

Aims: To examine the effects of clonidine on mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA) and vascular sensitivity to the selective α₁-adrenoceptor agonist phenylephrine (PE) and angiotensin II (Ang II) during hypotensive sepsis in conscious sheep.

Methods: Sepsis was induced in sheep by continuous administration of live E. coli (2.8 × 10⁹ bolus + 1.26 × 10⁹ colony forming units, i.v.) for 32 h. Pressor responses to increasing doses of PE and Ang II were assessed during baseline, 24, 28 and 32 h of sepsis following clonidine (n=6) or saline (n=6) treatment (1 μg/kg/h, from the 25th–32nd h of sepsis).

Results: Prior to treatment with clonidine or saline, sepsis was characterized by hypotension (~11 mmHg), along with elevations in HR (~90%), RSNA (~80%) and reduced pressor responses to PE and Ang II following 24 h of E. coli infusion. In saline-treated sheep, MAP progressively declined from the 25th to the 32nd h of sepsis, while the elevations in HR and RSNA and reduced pressor responsiveness to vasopressors were sustained (All *P* <0.01). In contrast, clonidine-treatment prevented the further decline in MAP and markedly reduced HR and RSNA (All *P*Interaction <0.001), and restored pressor responsiveness to both vasopressors back towards pre-septic levels (All *P*Time >0.05).

Conclusions: Administration of clonidine during hypotensive sepsis results in a better conservation of MAP, along with a significant reduction in HR and RSNA, indicating effective sympatho-inhibition. Clonidine-treatment also resulted in a striking restoration in vascular sensitivity to both PE and Ang II. Therefore, clonidine may be an effective therapeutic agent for treating human septic shock by improving the pressor responses to vasoconstrictor therapies, resulting in lower vasopressor requirements and thus better maintenance of arterial pressure and organ perfusion.

37: EFFECTS OF ANTI-HYPERTENSIVE TREATMENT ON FUNCTIONAL AND STRUCTURAL COMPONENTS OF LARGE ARTERY STIFFNESS AND RETINAL VESSEL DIAMETERS IN A RODENT MODEL OF TYPE I DIABETES

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Background: Diabetes is associated with cardiovascular risk, increased arterial stiffness, and ocular diseases. Whether large artery stiffness is independently associated with diabetes per se or concomitant hypertension is currently unknown.

Aims: To determine whether artery stiffness is independently associated with diabetes or is associated concomitantly with hypertension.

Methods: Male, Wistar rats (6 weeks of age) were divided into control (n=8), control with anti-hypertensive treatment (telmisartan, 10 mg/kg/day, n=8), induced diabetes (intraperitoneal streptozotocin, 50 mg/kg, confirmed by blood glucose measurement, n=12) and diabetes with anti-hypertensive treatment (n=12). At 18 weeks, rats were anaesthetized (urethane, 1.3 g/kg) and aortic pulse wave velocity (aPWV, aortic stiffness) was measured invasively across a full range of physiological arterial pressure (intravenous phenylephrine, sodium nitroprusside, 30 µg/kg/min). Retinal artery and venous diameters were measured using a custom microscope/camera assembly. Passive (elastin, collagen) and active (endothelial, smooth muscle function) components of aortic vessel stiffness were quantified using tensile testing and myography.

Results: Conscious, systolic blood pressure was high in both control and diabetic animals (142±16 and 132±22
mmHg, respectively) compared to control and diabetic animals on anti-hypertensive therapy (105±11 and 119±14 mmHg; P<0.01). Diabetic animals had marginally but significantly lower aPWV across all pressures. Anti-hypertensive treatment decreased aPWV within the low pressure range and increased aPWV within the high pressure range for both controls and diabetic animals. This resulted in increased pressure dependency of aPWV with anti-hypertensive treatment. Retinal venous diameters were greater with diabetes. Anti-hypertensive therapy increased retinal venous diameters but decreased arterial diameters. There was no difference in aortic endothelial dependent or independent vasorelaxation. Sensitivity to phenylephrine (vasoconstriction) was less in diabetic animals (P<0.05). Anti-hypertensive therapy caused a rightward shift in the aortic stress-strain curve (P<0.001).

**Conclusions:** Diabetes appeared to have a small but positive effect on arterial stiffness when studied independently of blood pressure. However, high blood pressure decreased the artery’s ability to respond to acute pressure changes, possibly due to remodelling of passive aortic wall components. Retinal venous diameters were greater with diabetes, with anti-hypertensive therapy having different effects on the retinal arteries and veins.

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**AVBS Session - IMMUNE MECHANISMS IN ATHEROSCLEROSIS**

### 38: DIFFERENTIAL EFFECTS OF B CELL SUBSETS IN ATHEROSCLEROSIS

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Atherosclerosis is a chronic inflammatory disorder responsible for the majority of deaths due to myocardial infarctions and strokes. Atherosclerotic lesions that develop in the vessel wall of medium/large arteries are the result of complex interactions between accumulated LDL-cholesterol, endothelial and smooth muscle cells and cells of the innate and adaptive immune system. Multiple immune cells accumulate in atherosclerotic lesions including macrophages and dendritic cells, NK and NKT cells, CD4+ and CD8+ T cells and B cells. Early studies indicated that B cells are protective against atherosclerosis by producing low affinity IgM antibodies against oxidised LDL (low density lipoprotein). However, recent advances in B cell biology indicate multiple subtypes of B cells suggesting a more complex role in the pathogenesis of atherosclerosis. Using an anti-CD20 B cell depletion antibody, we reinvestigated the role of B cells in atherosclerosis using fat fed ApoE-/- mice. Using such mAbs we demonstrated that B cell depletion decreases development and progression of murine atherosclerosis. Adoptive transfer approaches demonstrated that the B2 B cell subtype was proatherogenic. By targeting the B2 B cells using BAFF receptor knockout mice and anti-BAFF receptor antibodies we demonstrated their important role in atherosclerotic lesion inflammation and development/progression of atherosclerosis. Unlike B2 B cells, B1a B cells produce natural IgM antibodies as well as interleukin-10. In contrast to B2 B cells, deletion of peritoneal and splenic B1a B cells aggravates atherosclerosis. These effects were accompanied by marked reductions in anti-oxidised LDL IgM antibodies, lesion apoptotic cell numbers and necrotic core development. TLR4-MyD88 signaling is required for B1a B cell mediated suppression of atherosclerosis. Furthermore, targeting TIM-1 expressed by B1a B cells by administering anti-TIM-1 (RMT1-10) mAb to hyperlipidemic ApoE-/- mice expands the B1a B cell population including TIM-1+IgM+ and TIM-1+IgM+IL-10+ B1a B cell subsets and attenuates atherosclerosis.

### 39: MONOCYTE PRODUCTION IN DIABETES AND THE CONTRIBUTION TO ATHEROSCLEROSIS

**Murphy, Andrew**1, Kraakman, Michael1, Masters, Seth2, Dragoljevic, Dragana1, Nagareddy, Prabhakara3, Goldberg, Ira4

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An increased level of circulating monocytes is associated with atherosclerotic cardiovascular disease and can play a causative role. In the setting of diabetes, even when optimal lipid control is achieved, atherosclerotic lesion regression is impaired. We discovered that hyperglycemia drives monocyte production in mouse models of type 1 diabetes by stimulating haematopoietic progenitor cell proliferation. This increase in circulating monocytes contributed to impaired arterial regression as there was persistent entry of monocytes into the lesion. Interestingly, monocytosis is also observed in people with obesity/insulin resistance. We found that lowering blood glucose with a Sodium Glucose co-transporter 2 inhibitor (SGLT2i) in obese insulin resistant mice with mild hyperglycemia, failed to lower blood monocytes. Instead we found a prominent role for the obese adipose tissue (AT) in stimulating monocyte production. Adipose S100A8/A9 induced AT macrophage (ATM) TLR4/MyD88 and NLRP3 inflammasome-dependent IL-1β production. IL-1β interacted with the IL-1 receptor on BM myeloid progenitors to stimulate the production of monocytes and neutrophils. These studies uncover a positive feedback loop between ATMs and BM myeloid progenitors and suggest that inhibition of TLR4 ligands or the NLRP3-IL-1β signaling axis could reduce AT inflammation and insulin resistance in obesity.
40: ON THE TRAIL OF ATHEROSCLEROSIS

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Proliferation and apoptosis are intimately coupled processes in normal development, and in disease. TRAIL (tumour necrosis factor (TNF)-related apoptosis-inducing ligand) is a protein discovered only 20 years ago, that promotes apoptosis by binding to specific ‘death receptors’. TRAIL has since been the topic of >5000 studies. We have reported important novel functions for TRAIL including its ability to promote vascular cell proliferation and migration in vitro and in vivo. Our findings have established a protective function for TRAIL in cardiovascular disease (CVD), diabetes and nephropathy, such that TRAIL-deficient mice displayed accelerated disease with increased inflammation in blood vessels, pancreas and kidney, in response to a high fat Western style diet. Our more recent work demonstrates that TRAIL-deficient vessels are insulin resistant and this is associated with marked increases in inflammatory cytokine expression. Furthermore, we show that insulin differentially regulates TRAIL expression to modulate VSMC proliferation and/or apoptosis. This mechanism may therefore offer novel therapeutic solutions to combat diabetes induced CVD.

41: THE MULTIPLE ROLES OF CHEMOKINES IN ATHEROSCLEROSIS

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Chemokines constitute a family of structurally related chemotactic cytokines that direct the trafficking and functional regulation of leukocytes in normal conditions and in diverse pathologies. They are divided into four main sub-groups based on the position of the first two cysteine residues (e.g. CC, CXC, C, CX3C). In atherosclerosis, chemokines play key roles at each stage in development including monocyte recruitment, rolling interactions/arrest, monocyte trans-endothelial migration along a chemokine gradient, smooth muscle cell migration and macrophage emigration. Furthermore, the chemokine CXCL12 can promote regenerative processes following vascular injury via the mobilisation and recruitment of endothelial progenitor cells. The importance of chemokines in atherosclerosis has been identified using various strategies including murine chemokine knockout models and broad-spectrum chemokine inhibitors, which will be highlighted in this presentation. Furthermore, evidence that the interruption of chemokine/chemokine receptor interactions may be a suitable approach to treat atherosclerosis will be presented.

42: THERAPEUTIC ROLE OF TLR9 AGONIST IN THE ATHEROSCLEROSIS

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Background: Atherosclerosis is driven by inflammatory reactions that are shared with the innate immune system. Toll-like receptor-9 (TLR9) is an intracellular pattern recognition receptor of the innate immune system that is currently under clinical investigation as a therapeutic target in inflammatory diseases. Here we investigated whether TLR9 has a role in the development of atherosclerosis in ApoE-/- mice.

Methods and Results: Newly generated double-knockout ApoE-/-:TLR9-/- mice and control ApoE-/- mice were fed a high-fat diet from 8 weeks of age and effects on lesion size, cellular composition, inflammatory status, and plasma lipids were assessed after 8, 12, 15 and 20 weeks. All four time points demonstrated exacerbated atherosclerotic lesion severity in ApoE-/-:TLR9-/- mice, with a corresponding increase in lipid deposition and accumulation of macro-phages, dendritic cells and CD4+ T cells. Although ApoE-/-:TLR9-/- mice exhibited an increase in plasma VLDL/LDL cholesterol, the VLDL/LDL:HDL ratio was unaltered because of a parallel increase in plasma HDL cholesterol. As a potential mechanism accounting for plaque progression in ApoE-/-:TLR9-/- mice, CD4+ T cell accumulation was further investigated and depletion of these cells in ApoE-/-:TLR9-/- mice significantly reduced lesion severity. As a final translational approach, administration of a TLR9 agonist (type B CpG oligodeoxy–nucleotide ODN-1668) to ApoE-/- mice resulted in a reduction of lesion severity.

Conclusions: Genetic deletion of the innate immune receptor TLR9 exacerbated atherosclerosis in ApoE-/- mice fed a high-fat diet. CD4+ T-cells were identified as potential mediators of this effect. A type B CpG ODN TLR9 agonist reduced lesion severity, thus identifying a novel therapeutic approach in atherosclerosis.
Aging is determined by genetic and environmental interactions. Recent advances in aging research have revealed that cellular aging is a complex process involving 1) genome instability, 2) telomere consumption, 3) epigenetic changes, 4) protein instability, 5) nutrition sensing dysfunction, 6) mitochondrial dysfunction, 7) cell senescence, 8) stem cell depletion, 9) cell network disruption. Vascular aging with impaired angiogenesis is a major clinical problem in aged patients that often leads to ischemic peripheral artery and cardiovascular diseases.

Recent studies have focused on strategies to improve aged cells’ angiogenesis capability and subsequent tissue repair. Our recent studies have focused on both epigenetic modifications and protease regulation of the aged arteries. Our latest findings in vascular aging will be discussed, including microRNA-34a and cathepsin L regulations of angiogenesis in aged mice. The molecular imprints revealed including inhibition of the GTPCH1/tetrahydrobiopterin (BH4) pathway and activation of the inflammasome, p66shc and TLR4/MyD88 signaling, resulting in excessive oxidative stress, decreased telomerase activity, and accelerated senescence. These new findings may provide a molecular basis for new modalities that could be developed to therapeutically rescue impaired angiogenesis in aged patients.

44: HIGH INTRALUMINAL PRESSURE INCREASES VASCULAR INFLAMMATION

Chin-Dusting, Jaye
Baker IDI Heart and Diabetes Institute, Melbourne, Australia

Despite the widespread availability of blood pressure lowering medications, high blood pressure (high BP; hypertension; HT) remains responsible for more deaths and disease globally than any other biomedical risk. While numerous studies identify HT as a potent contributing factor in the development of coronary artery disease (CAD), the exact mechanism by which this occurs is not known. We have novel evidence that high intraluminal pressure per se causes leukocyte to endothelium adhesion, a hallmark of vascular inflammation, and thus forward the hypothesis that high pressure induces inflammation which can result in plaque formation and progression.

We show for the first time that high pressure alters caveolae structure and function and suggest that this structural disruption generates increased oxidative stress, upregulates NFκB and increased expression of adhesion molecules and selectins responsible for the adhesion cascade and eventual plaque formation and progression. Furthermore, we show in a novel spontaneously hypertensive diet-induced atherosclerotic mouse model (BPHx Apoe−/− mice) that the increase in pressure renders aortic sinus lesions from mice with greater lipid deposition (Oil Red O; P<0.05) and macrophage content (CD68; P<0.05) compared to Apoe−/− mice, indicative of reduced plaque stability.

We conclude that high intraluminal pressure induces vascular inflammation by promoting caveolae flattening, NADPH oxidase dependent ROS production and NFκB translocation, which all contribute to endothelial activation, adhesion molecule expression and enhanced leukocyte adhesion leading to increased plaque instability.

45: THE MECHANISM BY WHICH MECHANICAL STRESS ACTIVATES AT1 RECEPTORS

Zou, Yunzeng
Zhongshan Hospital Fudan University Shanghai, China

In the heart, mechanical load is a crucial regulator of myocardial structure and function; however, mechanical overload is a pathogenesis or comorbidity existing in a variety of heart diseases, such as hypertension, aortic regurgitation and myocardial infarction. The angiotensin II (AngII) type 1 receptor (AT1-R) is a 7 transmembrane G protein-coupled receptor that plays a critical role in load-induced cardiac hypertrophy. Early studies revealed the involvement of autocrine/paracrine mechanisms through stretch-induced release of AngII. Recent conceptually inspiring studies unraveled that the AT1-R could be also directly activated by mechanical stress. The activated AT1-R initiates intricate intracellular signaling pathways through G protein-dependent and G protein-independent mechanisms. AT1-R blocker (ARB) antagonizes the activation of AT1-R to regress cardiac remodeling. Some ARBs show properties of inverse agonism at the AT1-R, which are potential therapeutic targets for the treatment of load-induced cardiac hypertrophy. We here summarize the progress in the understanding of ligand- and mechanical stress-dependent activation of AT1-R, highlight recent data that investigate the role of AT1-R in load-induced cardiac hypertrophy, and discuss the clinical relevance of inverse agonism of AT1-R ligand.
Thursday 27th November

08:00 - 08:45 PLENARY SESSION - MEETING WELCOME HALL D

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08:45 - 10:15 ISCP Session - Treating the Patient with AFIB RB 3

Chairpersons: George Dan, Margaret Arstall

46 08.45 Antoni Martinez-Rubio Prevention of stroke in atrial fibrillation: are all oral drugs the same?
47 09.05 George Dan Antiarrhythmic drugs for atrial fibrillation
48 09.30 Prash Sanders Ablation or other non-pharmacological management of AF
49 09.55 Antoni Martinez-Rubio Cost-effectiveness of oral anticoagulant drugs for stroke preventions in patients with non-valvular atrial fibrillation

08:45 - 10:15 AAS / AVBS / HBPRCA - Inflammation HALL D

Chairpersons: Connie Wong, Christina Bursill

50 08.45 Oliver Soehnlein Mechanisms of neutrophil-instructed monocyte recruitment
51 09.15 Kevin Woolard Exploring the functions of blood monocytes and responses to lipids
52 09.45 Shane Thomas Myeloperoxidase and its role as a cause of endothelial dysfunction during inflammatory vascular disease
53 10.00 Michael Hickey Regulating the endothelium - interactions of regulatory T cells and vascular endothelial cells

11:00 - 12:30 ISCP / HBPRCA Joint Session - Treating High Blood Pressure HALL D

Chairpersons: John McNeil, Ardunio Mangoni

54 11.00 John Chalmers Guidelines for the Management of High Blood Pressure
55 11.25 Bruce Neal Dietary salt and hypertension
56 11.45 Stephen Harrap Pharmacological Prevention of Hypertension
57 12.05 Thomas Kahan Treating high blood pressure: combination therapy

11:00 - 12:30 AAS Session - The Heart RB 1/2

Chairpersons: Rebecca Ritchie, Fadi Charchar

58 11.00 Enzo Porrello Epigenetic control of cardiac development and regeneration
59 11.20 Arthur Christopoulos Exploiting allosteric and biased receptor signalling in cardiovascular disease
60 11.40 Julie Mullen A novel therapy for restoring heart function and rhythm
61 12.00 Anisyah Ridlandres (student finalist) CC-chemokine class inhibition attenuates inflammatory induced pathological angiogenesis whilst preserving ischaemia driven physiological angiogenesis
62 12.15 Kwok Leung Ong Relationship of pericardial fat with biomarkers of inflammation and hemostasis, and cardiovasculardisease: The Multi-Ethnic Study of Atherosclerosis

11:00 - 12:30 AVBS Session - Angiogenesis and Lymphangiogenesis RB 3

Chairpersons: Marc Achen, Claudine Bonder

63 11.00 Veronique Angeli Lymphatic vessels in atherogenesis
64 11.30 Philip Hogg Regulation of vascular proteins by allosteric disulphide bonds
65 11.50 Natasha Harvey Understanding the mechanisms by which GATA2 mutations cause primary lymphoedema
66 12.10 Sophie Paquet-Fifield Regulation of lymphangiogenesis in cancer

13:30 - 14:00 ISCP INVITED LECTURE HALL D
Harvey White Targeting inflammatory and lipid pathways for the treatment of cardiovascular disease

14:00 - 15:30 ISCP / AVBS Session - Treating Patients with Coronary Disease HALL D

Chairpersons: Gemma Figtree, Stephen Nicholls

67 14.00 Karlheinz Peter Novel ways to inhibit platelets without bleeding complications
68 14.20 Phil Aylward Observational data / use of stabilised treatments
69 14.40 Juan Carlos Kaski Novel management of stable angina pectoris
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HENRY NEUFELD LECTURE

ENERGETIC/INFLAMMATORY DISTURBANCES IN HEART DISEASE: EMERGING THERAPEUTIC PERSPECTIVE

Professor John Horowitz

Myocardial ischaemia is associated with both redox stress and energetic depletion, and a number of anti-anginal agents have been shown to favourably affect myocardial metabolism to improve energetics. The therapeutic utility of this approach has recently been extended by the identification of myocardial energetic impairment associated with systolic and diastolic heart failure, hypertrophic cardiomyopathy, diabetes mellitus and Tako-Tsubo Cardiomyopathy. Furthermore, many biochemical pathways and enzymes which potentially can be manipulated therapeutically to improve energetics have been identified, including the Randle cycle, AMPkinase, PARP, thioredoxin-interacting protein (TxNIP) and the uncoupling proteins. Furthermore, many of the emerging agents which improve myocardial energetics also exert important effects as anti-inflammatory agents. The emerging spectrum of therapeutic utility of drugs which normalise myocardial energetics will be discussed.

ISCP Session - TREATING THE PATIENT WITH AFIB

46: PREVENTION OF STROKE IN ATRIAL FIBRILLATION: ARE ALL ORAL DRUGS THE SAME?

Martínez-Rubio, Antoni

Univ. Hospital of Sabadell, (Univ Autònoma of Barcelona), Department of Cardiology, Sabadell, Spain

Prevention of thromboembolic events using oral anticoagulant drugs (OAC) is mandatory in several patients with atrial fibrillation. Therefore, OAC have been developed and used since decades. However, efficacy of these must be well balanced with the risk of inherent bleeding complications. Dicumarine (DC-D) derivates are useful but need monitoring and show several food and drug interactions, which may be critical. Therefore, novel OAC (NOAC) have been developed. These new drugs interact with two different targets (factor IIa or factor Xa of the coagulation cascade).

Actually, the clinical results of four NOACS (dabigatran (DAB), rivaroxaban (RIV), apixaban (API), edoxaban (EDO)) have been presented in four major trials (RELY, ROCKET-AF, ARISTOTLE, ENGAGE-AF) of stroke prevention in atrial fibrillation in comparison with warfarin.

Pharmacokinetics and pharmacodynamics of the four NOACS are different. Thus, DAB and API are given twice/daily whereas RIV and EDO have been tested in once daily approach. In addition, renal clearance ranges from 25% with API to 80% with DAB (33% with RIV and 35% with EDO). Furthermore, bioavailability, hours to maximal concentration, CYP metabolism, transporters, protein binding and half-life also differ between NOACS.

Thus, plasma levels of NOACs may influence and also be influenced by some drugs, which are often used (e.g. diltiazem, amiodarone, etc.). Therefore, physicians need to know the main results of the four major clinical trials, those of important sub-studies and also the major differences between the four NOACS in order to manage properly the stroke preventive strategies of patients with atrial fibrillation. In addition, since NOACs have been used in different dosages during the trials, physicians should know which populations do benefit of low or high dosages of the NOACs and how to manage complications.

In resume, NOACs demonstrate several advantages over DC-D but physicians need to know how to implement treatment with these new drugs and which are the main results and characteristics of all of them.

47: ANTIARRHYTHMIC DRUGS FOR ATRIAL FIBRILLATION

Dan, George

Bucharest, Romania

Despite the increasing interest in treating atrial fibrillation (AF) the pharmacologic treatment aimed to maintain normal sinus rhythm (SR) represents a huge challenge both for present and the future. The development of the interventional therapy was not followed by a parallel development of drugs despite the fact that pharmacological therapy or arrhythmias could not be replaced by the first. The CAST study showed that efficient drugs in treating arrhythmias could increase mortality and the AFFIRM study found no differences between rate and rhythm strategy because improvement of prognosis with SR is counterbalanced by negative effect of the classic antiarrhythmic drugs (AAD). The Singh-Vaughan-Williams classification of AAD is a too narrow and rigid box for the complex understanding of both the electrophysiological and clinical effects of AAD. Better characterization of the arrhythmia mechanism and venerable parameters together with the potential implication for proarrrhythmias and organ toxicity represent the clinical basis for introducing new AAD in practice. AF is initiated by and initiates a complicated electrical, functional and structural remodeling evolving process. Efforts aimed in preserving SR should interfere intimately during this process. Three main directions characterize the development of new AAD for AF. First, the so called “upstream therapy” is directed against the substrate remodeling; these nonconventional AAD have important implications in primary prevention and perpetuation of AF. Novel targets in this direction (i.e. calcium signaling molecules) are under evaluation. Modern
AAD are targeting excitability, ectopic activity and complex reentry. These new drugs are developing either exploiting some of the properties of the old class III AAD, especially amiodarone or targeting atrial specific or selective sarcolemmal or intracellular channels.

49: COST-EFFECTIVENES OF ORAL ANTICOAGULANT DRUGS FOR STROKE PREVENTIONS IN PATIENTS WITH NON-VALVULAR ATRIAL FIBRILLATION

Martínez-Rubio, Antoni
Univ. Hospital of Sabadell, (Univ Autònoma of Barcelona), Department of Cardiology, Sabadell, Spain

Prevention of thromboembolic events using oral anticoagulant drugs (OAC) is mandatory in several patients with atrial fibrillation. Therefore, OAC have been developed and used since decades. Efficacy of anticoagulant drugs must be well balanced with short and long-term safety. Dicumarine (DC-D) derivates are useful but need chronic monitoring of plasmatic levels because they present a narrow therapeutic window, show several food and drug interactions as well as genetic variants which may be critical for the long-term safety of patients. To avoid DC-D limitations, novel OAC (NOAC) have been developed. Actually, the clinical results of four NOACS (dabigatran, rivaroxaban, apixaban and edoxaban) have been presented in four major trials (RELY, ROCKET-AF, ARISTOTLE, ENGAGE-Af) of stroke prevention in atrial fibrillation in comparison with warfarin.

During this presentation, the clinical usefulness of NOACs will be compared with dicumarine derivates and basic economic considerations will be reviewed. Thus, the purpose of this presentation is the introduction to Pharmacoeconomy for physicians (based in the interpretation of clinical data obtained from the most recent major anticoagulation trials in atrial fibrillation) for understanding the economic impact and repercussions of any clinical decision of use.

50: MECHANISMS OF NEUTROPHIL-INSTRUCTED MONOCYTE RECRUITMENT

Soehnlein, Oliver 1-3
1Institute for Cardiovascular Prevention (IPEK), Ludwig Maximilian University (LMU) Munich, Germany; 2Academic Medical Center (AMC), Department of Pathology, Amsterdam University, the Netherlands; 3DZHK, partner site Munich Heart Alliance, Germany.

The leukocyte response in acute inflammation is characterized by an initial recruitment of neutrophils preceding a second wave of monocytes. Preformed neutrophil-derived granule proteins with chemotactic ability were suggested to hold an important role in this cellular switch. In the recent years we could show that neutrophil-borne cathelicidins (LL37 in human, CRAMP in mouse) are deposited at sites of inflammation and chemoattract classical monocytes via engagement of FPR2. Such mechanism is not just important in acute inflammatory responses but also in atherosclerosis, where cathelicidins contribute importantly to early macrophage accumulation. Interestingly, platelets exert very similar activities, releasing preformed chemokines that induce adhesion and migration and monocytes. In very recent studies we could evidence a cooperation of neutrophils and platelets in promotion of monocyte recruitment. Herein, granule proteins released from platelets and neutrophils form heteromers which potently stimulate classical monocyte adhesion.

51: EXPLORING THE FUNCTIONS OF BLOOD MONOCYTES AND RESPONSES TO LIPIDS

Woollard, Kevin
Division of Immunology & Inflammation, Department of Medicine, Imperial College London, United Kingdom

Monocytes are traditionally thought as precursors to tissue macrophages. However, recent data shows this model may not be as clear in all organs and tissues, due to the description of embryonic origins of macrophages which can proliferate in-situ and do not need replenishment by blood monocytes. Therefore important questions include, do blood monocytes have distinct effector functions and what is their specific role in health, immunity and vascular disease? We are developing complementary imaging and molecular techniques to try and characterise the heterogeneous behaviour and effector functions of monocytes in-vitro and in-vivo.

In humans, three circulating monocyte subsets can be identified based on CD14 and CD16 expression and at least 2 subsets in mice based on Ly6c/Gr1 expression. CD14highCD16low (Ly6c/Gr1high) monocytes are well-characterized inflammatory monocytes that respond to bacterial cues, while CD14lowCD16high (Ly6c/Gr1low) monocytes can crawl on the vascular endothelium in the steady state, although their role in homeostatic conditions is largely unresolved.
CD14<sup>high</sup>CD16<sup>high</sup> are poorly described in mice, but are shown to have increased frequency in human inflammatory diseases. Unstable cardiovascular disease (CVD) still remains a leading cause of death worldwide. A major risk factor for CVD is elevated lipid, which include oxidative modified low-density lipoproteins (mLDL) and triglyceride-rich lipoproteins (TGRL). Experimental models show that mLDL and TGRL in atherosclerotic prone vascular beds leads to increased monocyte recruitment, macrophage foam cell formation and atherosclerosis. However, with the increasing appreciation of resident tissue macrophage self-renewal and blood monocyte functional heterogeneity, this described model has been complicated. Importantly, the subset specific effector functions of blood monocytes in dyslipidaemia and CVD remain poorly described.

In summary, we would like to study the sentinel activity of blood monocytes by describing the subset specific effector functions that include their behaviour at the endothelial interface, TLR responses, anti-microbial phenotype and phagocytic activity, together with further detailed descriptions of their behaviour in dyslipidaemic environments and therefore potential consequences for immunity and CVD progression.

52: MYELOPEROXIDASE AND ITS ROLE AS A CAUSE OF ENDOTHELIAL DYSFUNCTION DURING INFLAMMATORY VASCULAR DISEASE

Thomas, Shane R.
Centre for Vascular Research, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia.

Considerable clinical and experimental evidence implicate a multi-faceted role for the leukocyte-derived, oxidative stress enzyme myeloperoxidase (MPO) during inflammatory cardiovascular disease. Elevated levels of circulating, extracellular MPO are a feature of cardiovascular disease patients and represents an independent predictor of the prevalence of coronary artery disease and clinical event risk in patients with acute coronary syndrome. Circulating, extracellular MPO released by activated leukocytes can exit the circulation by binding to the luminal surface of endothelial cells in a heparan sulfate-dependent manner, undergoing transcytosis across the endothelium and accumulating within the sub-endothelial space of arteries. At this site, endothelial-localized MPO catalyzes local oxidative reactions capable of promoting endothelial dysfunction, including the enzyme’s ability to produce the reactive oxidant hypochlorous acid (HOCl) or catalytically consume endothelial-derived nitric oxide (NO) via its NO oxidase activity. This presentation will detail our recent in vitro, ex vivo and in vivo studies into the oxidative reactions and molecular signalling mechanisms by which extracellular MPO can promote endothelial dysfunction and augment inflammatory artery disease. Also discussed, will be our studies into therapeutic strategies aimed at targeting extracellular MPO during inflammatory cardiovascular disease.

53: REGULATING THE ENDOTHELIUM – INTERACTIONS OF REGULATORY T CELLS AND VASCULAR ENDOTHELIAL CELLS

Hickey, Michael J. 1, Abeynaike, Latasha D. 1, Westhorpe, Clare L.V. 1, Deane, James A. 1
1Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Clayton, Melbourne, Victoria, Australia

Regulatory T cells (Tregs) are a subset of CD4<sup>+</sup> T lymphocytes that limit inflammation in peripheral tissues. Studies of the skin show that in order for Tregs to inhibit inflammation, they must be able to undergo interactions in the dermal microvasculature. Despite this, little is known regarding the interactions whereby these cells undergo recruitment. Moreover, despite the identification of numerous potential mechanisms used by Tregs to limit inflammation, exactly how they achieve this function in vivo remains unclear. Therefore, the aim of these studies was to examine Treg trafficking in a model of dermal inflammation, using confocal intravital microscopy to examine Treg-endothelial interactions.

Foxp3-GFP mice were used to enable visualisation of Tregs in vivo. Tregs underwent rolling interactions on the endothelium, although these were minimally altered during inflammation. Treg rolling was generally mediated by endothelial P- and E-selectin in tandem, although after a second hapten challenge, rolling of Tregs but not conventional CD4<sup>+</sup> T cells became P-selectin-independent. E-selectin inhibition at this phase of the response led to increased skin inflammation consistent with the notion that having the capacity to interact with the endothelium is critical in the ability of Tregs to regulate skin inflammation.

Tregs were also observed to undergo adhesion in the inflamed dermal microvasculature, representing ~40% of the total adherent CD4<sup>+</sup> T cells at the peak of the response. Time lapse observations of adherent Tregs revealed that at a certain time point in the response, Tregs underwent prolonged intravascular crawling without undergoing transmigration. At this point, elimination of Treg adhesion via ICAM-1 inhibition resulted in increased adhesion of pro-inflammatory leukocytes. Together these observations demonstrate the complexity of Treg-endothelial cell interactions during a T cell-mediated inflammatory response, and indicate that one mechanism whereby Tregs control inflammation in the periphery is via regulation of endothelial cell adhesive function.
54: GUIDELINES FOR THE MANAGEMENT OF HIGH BLOOD PRESSURE

Chalmers, John
The George Institute for Global Health, The University of Sydney, NSW, Australia.

There have been many international Guidelines issued in the past 2 years, with considerable consistency, overshadowed by controversy and disagreement over some key issues. In this presentation, I will discuss three issues:

- The process of guideline development, with focus on the restrictions imposed by the avoidance of conflicts of interest and the need to limit recommendations to those supported by solid evidence.
- The disagreement over blood pressure targets, especially with respect to age.
- The disagreement over the choice of drug classes for the initiation of drug treatment.

55: DIETARY SALT AND HYPERTENSION

Neal, Bruce
The George Institute for Global Health, Sydney, Australia

Salt reduction has been identified as a public health priority but there are few data describing the effects of plausible salt reduction programs on population salt consumption. A multi-faceted, community-based salt reduction program using the Communication for Behavioral Impact framework was implemented in Lithgow, Australia between 2011 and 2013. 24-hour urine samples were obtained from a large number of individuals at baseline and follow-up, as was information about knowledge and behaviors relating to salt. The effects on mean salt intake estimated from 24-hour urinary samples will be reported as will the effects on knowledge and behaviors. The implications of the program will be reviewed in light of current evidence about the likely effects of salt reduction on cardiovascular health.

56: PHARMACOLOGICAL PREVENTION OF HYPERTENSION

Harrap, Stephen
Department of Physiology, University of Melbourne, Melbourne, Australia

The Spontaneously Hypertensive Rat (SHR) is well known as a model of human essential hypertension. Numerous independent studies have shown that inhibitors of the renin-angiotensin system (RAS) (typically ACEi or angiotensin receptor blockers) administered in the prehypertensive period (typically from 6 to 10 weeks of age) to SHR result in a permanent reduction in blood pressure and prolongation of life. These persistent effects of RAS inhibition are not simply a non-specific effect of blood pressure reduction during the pre-hypertensive phase, as they are not observed in SHR following treatment with vasodilators (eg hydralazine) or calcium antagonists.

Not all inbred hypertensive rat strains mimic SHR, which implies that distinct genetic mechanisms exist in the SHR that program the long-term response to RAS inhibition. The prehypertensive SHR has abnormally increased renal vascular resistance sufficient to compromise renal blood flow and glomerular filtration rate. These renal hemodynamic abnormalities in SHR respond acutely to RAS inhibition. The importance of the kidney in SHR is further emphasized by the results of renal transplantation studies. The blood pressure “follows the kidney” in cross-transplantation experiments between previously RAS-inhibited and untreated SHR. At least 3 trials have attempted to emulate SHR responses in humans (TROPHY, DHyPP and STAR CAST) but have met with mixed success. The future of pharmacological prevention of hypertension is real but needs further refinement.

57: TREATING HIGH BLOOD PRESSURE: COMBINATION THERAPY

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Although the efficacy of antihypertensive drug therapy is undisputed, observational studies show that few patients reach target blood pressure. Most antihypertensive drug classes reduce blood pressure by approximately 10/5 mmHg, when corrected for placebo. Thus, most patients will be expected to need two or more drug classes to achieve target blood pressure. Preferred drugs are those with a proven efficacy on cardiovascular outcome. Several well-known combinations of antihypertensive drugs are well documented. Drug combinations should represent different mechanisms of action to provide additive or synergistic effects.
Angiotensin converting enzyme inhibitors and angiotensin receptor blockers are together with calcium channel blockers and thiazide like diuretics often considered first antihypertensive drug classes. They can all be combined. However, the addition of dihydropyridine type calcium channel blockers to blockers of the renin-angiotensin-aldosterone system may have an advantage over adding diuretics. The combination of beta-adrenergic blockers and diuretics may promote glucose intolerance; the clinical implications of this are, however, not yet settled. The addition of alpha-adrenergic blockers, mineralcortcoid recepto antagonists and centrally acting drug classes have additional blood pressure lowering effects but effects on cardiovascular outcome in hypertension are not yet proven.

Patient preferences, potential risk in the individual patient for side effects, and concomitant conditions and diseases should guide the decision in antihypertensive drug combination therapy.

AAS Session - THE HEART

58: EPIGENETIC CONTROL OF CARDIAC DEVELOPMENT AND REGENERATION

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Epigenetic modifications have recently emerged as central players in the coordination of gene expression networks during cardiac development. Much attention has focused on the role of histone modifications during embryonic heart development, but relatively little is known about the epigenetic control mechanisms that guide post-natal heart maturation. Furthermore, few studies have investigated the role of DNA methylation during cardiac development, despite the fundamental importance of this biological process for transcriptional regulation. The purpose of the current study was to determine whether DNA methylation plays an important role in guiding transcriptional changes during the neonatal period, which is an important developmental window for cardiac maturation, including cardiomyocyte cell cycle withdrawal and loss of endogenous cardiac regenerative capacity. Here, we provide novel evidence for widespread alterations in DNA methylation during post-natal heart maturation. Furthermore, our studies suggest that DNA methylation plays an important role in the transcriptional silencing of key regulatory networks for muscle development and cardiomyocyte proliferation during neonatal life.

59: EXPLOITING ALLOSTERIC AND BIASED RECEPTOR SIGNALLING IN CARDIOVASCULAR DISEASE

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G protein-coupled receptors (GPCRs) are the largest class of drug targets. They adopt multiple active states that can be differentially stabilized by both canonical orthosteric ligands and structurally distinct allosteric ligands—a phenomenon referred to as “biased agonism”. The promise of biased agonists as therapeutic agents is the potential to promote beneficial physiological effects while avoiding side-effects that are themselves mediated by the same target. For example, the adenosine A1 GPCR (A1AR) is an important therapeutic target for cardioprotection, but current agents acting on the receptor are clinically dose-limited due to on-target bradycardia. Through the rational design of a “bitopic” (hybrid orthosteric/allosteric) ligand, we have overcome this limitation to yield a biased agonist that protects against ischemic insult in native A1AR expressing cells and intact hearts without affecting heart rate, providing proof of concept for GPCR allosteric modulation and biased agonism as novel avenues for cardiovascular drug discovery.

60: A NOVEL THERAPY FOR RESTORING HEART FUNCTION AND RHYTHM

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Heart failure (HF) and atrial fibrillation (AF) frequently coexist and are associated with high mortality. Treatment of HF with AF represents a major unmet need. The objective of this study was to assess the therapeutic potential of a small molecule called BGP-15 in two mouse models which develop HF and AF. BGP-15 improved cardiac function and reduced arrhythmic episodes, and this was associated with increased phosphorylation of the insulin-like growth factor 1 receptor (IGF1R). Cardiac-specific IGF1R transgenic overexpression in mice with HF and AF mimicked the protection observed with BGP-15. Both BGP-15 and IGF1R provided protection independent of phosphoinositide 3-kinase-Akt and heat shock protein 70; signaling mediators often defective in the aged and diseased heart. Since BGP-15 is safe and well-tolerated in humans, this study uncovers a potential therapeutic approach for HF and AF.
61: CC-CHEMOKINE CLASS INHIBITION ATTENUATES INFLAMMATORY INDUCED PATHOLOGICAL ANGIOGENESIS WHILST PRESERVING ISCHAEMIA DRIVEN PHYSIOLOGICAL ANGIOGENESIS

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Introduction: Angiogenesis is critical for survival and in the regenerative response to tissue hypoxia. However, an imbalance in its regulation causes excessive angiogenesis, exacerbating inflammatory diseases such as cancer and atherosclerosis. Increasing evidence suggests the CC-chemokine class promote inflammatory-driven angiogenesis but, in contrast, have no role in hypoxia-driven angiogenesis. Inhibition of the CC-chemokine class may therefore regulate angiogenesis differently depending on the pathophysiological context.

Aim: Using the broad-spectrum CC-chemokine inhibitor ‘35K’, compare the role of the CC-chemokine class in inflammatory versus ischaemia driven angiogenesis.

Method and Results:PBS or adenoviruses expressing GFP (AdGFP) or 35K (Ad35K) were injected into mice 3 days before surgery. Using the murine femoral cuff model of inflammatory angiogenesis, we found a significant decrease in adventitial CD31positive (39.7%) and a-actin positive (75.5%) neovessels in the Ad35K group compared to the AdGFP controls, p<0.05. Ad35K mice also had significantly less adventitial macrophages (CD68, 32.5%). In contrast, 35K had no effect on neovascularisation in the context of hypoxia in the murine hind limb ischaemia model. There were no changes between treatment groups in blood flow recovery as measured using laser Doppler imaging and no changes in capillary or arteriole density in the gastrocnemius muscle. Consistent with our in vivo findings, in vitro angiogenic assays revealed that 35K protein had inhibitory effects on inflammation-induced endothelial cell Matrigel tubule formation (95.8%), proliferation (88.3%) and migration (15.8%), p<0.05. In hypoxia the inhibitory effects of 35K were more modest for tubule formation (45.2%) and proliferation (43.8%), and migration was completely preserved. Furthermore, under inflammatory conditions 35K inhibited vascular endothelial growth factor (VEGF, 53.4%) and hypoxia inducible factor-1α (HIF-1α, 87.3%) endothelial cell protein levels, yet in hypoxia 35K had no effect on VEGF and had a more modest inhibitory effect on HIF-1α (65.4%).

Conclusion: Broad-spectrum CC-chemokine inhibition by 35K inhibits inflammation-induced angiogenesis, whilst preserving ischaemia-driven angiogenesis in vitro and in vivo. CC-Chemokine inhibition may be a therapeutic strategy to reduce pathological angiogenic diseases without the severe side effects of current therapies caused by complete inhibition in both contexts.

62: RELATIONSHIP OF PERICARDIAL FAT WITH BIOMARKERS OF INFLAMMATION AND HEMOSTASIS, AND CARDIOVASCULAR DISEASE: THE MULTI-ETHNIC STUDY OF Atherosclerosis

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Background and Aims: Pericardial fat may increase the risk of cardiovascular disease (CVD) by increasing circulating levels of inflammation and hemostasis biomarkers. We investigated the associations of pericardial fat with inflammation and hemostasis biomarkers, as well as incident CVD events, and whether there are any ethnic differences in these associations.

Methods: The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal cohort, consisting of 6814 men and women in four major ethnic groups, non-Hispanic whites, African American, Hispanic American, and Chinese Americans. All participants were between 45 and 84 years of age and free of clinically apparent CVD at baseline (47.2% male, 38.4% Caucasian, 27.5% African American, 22.1% Hispanic American and 12.0% Chinese American). We analyzed results from 6,415 MESA participants who had measurements of pericardial fat volume and circulating levels of C-reactive protein (CRP), fibrinogen, interleukin (IL)-6, factor VIII, D-dimer and plasmin-antiplasmin complex (PAP), and had a mean follow-up period of 9.5 years. CVD event was defined as myocardial infarction, resuscitated cardiac arrest, angina, stroke, stroke death, coronary heart disease death, other atherosclerotic death, and other CVD death.

Results: After adjusting for confounding factors (including age, sex, ethnicity, body mass index, education, smoking, pack-years of smoking, current alcohol use, total gross family income, physical activity, heart rate, diabetes, hypertension, dyslipidemia and biomarkers of inflammation and hemostasis), pericardial fat volume was positively associated with natural log (ln) of IL-6 levels, but inversely associated with ln D-dimer and ln PAP levels (β=0.067, -0.032, and -0.105 respectively, all P<0.05). Although a larger pericardial fat volume was associated with a higher risk of incident CVD, the association was attenuated to borderline significance after adjusting for traditional cardiovascular risk factors (P=0.050). There was a borderline significant ethnicity interaction (P=0.080), whereby the association between pericardial fat and incident CVD was significant in Hispanic Americans, even after further adjusting for biomarkers of inflammation and hemostasis (hazard ratio=1.31 per SD increase, P=0.004).

Conclusions: Pericardial fat was associated with several inflammation and hemostasis biomarkers. The association of pericardial fat with incident CVD events was independent of these biomarkers only among Hispanic Americans.
63: LYMPHATIC VESSELS IN ATHEROGENESIS

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It became apparent from emerging research that lymphatic vessel can play a more extensive role in lipid metabolism than previously realized. Recently, we demonstrated that lymphatic vessels are essential for reverse cholesterol transport (RCT), a process mediated by high-density lipoprotein (HDL) which prevents excess cholesterol in tissues. We showed that removal of cholesterol by lymphatics is dependent on the uptake and transcytosis of HDL by scavenger receptor class B type I. We have previously shown that hypercholesterolemia in apoE-/- mouse is associated with impaired lymphatic drainage and increased lipid accumulation in peripheral tissues. We now show that restoring lymphatic drainage in these mice without affecting hypercholesterolemia significantly improves cholesterol clearance. Since RCT protects against atherogenesis, this novel role of lymphatic vessels in RCT may connect lymphatic function to atherosclerosis. Consistent with recent studies, our preliminary data in apoE-/- mice support the concept that poor lymph flow may contribute to atherosclerosis.

64: REGULATION OF VASCULAR PROTEINS BY ALLOSTERIC DISULPHIDE BONDS

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Most proteins are chemically modified after they are made in order to control how, when, and where they function. It was thought that only the amino acid side chains and the peptide bonds of proteins were chemically modified. We now know that the disulphide bonds of proteins are also modified. Some disulphide bonds are cleaved in the mature protein to control function: these are known as ‘allosteric disulphides’. There are more than 30 well defined examples of allosteric disulphides and many more that are under investigation. The indications are that cleavage of disulphide bonds may rival proteolysis of peptide bonds as a means of protein control.

About half the allosteric bonds identified to date reside in circulating proteins and 16 different disulphide bond reductases, the enzymes that cleave the allosteric bonds, have been identified in plasma and/or on the surface of platelets or leukocytes. The first four reductases investigated have been shown to function in thrombosis or inflammation. It is apparent that there is another level of regulation of circulating proteins that we are just beginning to understand. I will highlight an allosteric disulphide that controls the haemostatic activity of the blood protein, von Willebrand factor (VWF).

VWF is a multimeric protein that tethers platelets to the injured vessel wall during thrombosis. Only the largest multimers are effective at capturing platelets in the shear forces of flowing blood. VWF multimeric size is regulated in the circulation by proteolysis of the A2 domain Tyr1605-Met1606 peptide bond by ADAMTS13. The A2 domain contains an unusual disulphide bond that links adjacent cysteine residues Cys1669 and Cys1670. Mass spectrometry analysis of human plasma VWF indicates that the disulphide bond is naturally reduced in about one in two VWF subunits. The reduced VWF is much more efficiently cleaved by ADAMTS13 than the oxidised protein. The disulphide bond has a standard redox potential of -283 mV and MD simulations revealed that reduction of the bond has a pronounced effect on the structure, dynamics and internal stress of the domain. The redox state of the VWF A2 domain bond was examined in patients with unexplained bleeding who have normal VWF antigen/function levels, normal platelet aggregation and no known bleeding diathesis or thrombocytopenia. The patient’s VWF was more reduced than normal, which is consistent with the bleeding.

These findings imply that the Cys1669-Cys1670 bond acts as an allosteric switch in VWF by controlling ADAMTS13 regulation of the A2 domain bond.

65: UNDERSTANDING THE MECHANISMS BY WHICH GATA2 MUTATIONS CAUSE PRIMARY LYMHOEDEMA

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Lymphatic vessels are vital for tissue fluid homeostasis, the absorption of dietary fats and immune cell trafficking. Despite the integral role that lymphatic vessels play in homeostasis and human disease, little is known about the mechanisms that direct construction of the lymphatic vasculature during development. We and others recently reported that mutations in GATA2 underlie Emberger Syndrome, a disorder characterised by primary lymphoedema and myelodysplastic syndrome (MDS)/acute myeloid leukaemia (AML). Primary lymphoedema occurs as a result of aberrations in the development and/or function of lymphatic vessels, suggesting to us that GATA2 plays a key role in the lymphatic vasculature. Here, we demonstrate that GATA2 protein is present at high levels in lympho-venous and lymphatic vessel valves from the onset of valve morphogenesis in the mouse embryo. Moreover, we reveal that GATA2 controls the expression of genes important for the development of lymphatic vascular valves, in particular the transcription factors Prox1 and Foxc2. Conditional deletion of Gata2 in the lymphatic vasculature of mice disrupted lymphatic vessel valve morphogenesis and halted vessel maturation, demonstrating cell autonomous roles for Gata2 in lymphatic endothelial cells. Moreover, heterozygous Gata2 mutant mice exhibited lymphatic phenotypes including aberrant lymphatic vessel patterning and defective lymphatic vascular transport. Current work aims to determine the molecular mechanisms by which GATA2 controls gene expression in the lymphatic vasculature, in order to understand precisely how GATA2 mutations cause human lymphoedema.

**66: REGULATION OF LYMPHANGIOGENESIS IN CANCER**

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The secreted vascular endothelial growth factors VEGF-C and VEGF-D are soluble glycoproteins that induce the growth of blood vessels and lymphatic vessels and promote solid tumor growth and distant organ metastasis. VEG-C and VEG-F-D induce angiogenesis and lymphangiogenesis via binding to VEG receptor (VEGFR)-2 and 3. Both VEG-F-C and VEG-F-D are secreted as full length proteins, consisting of propeptides flanking the central VEGF homology domain. Proteolytic cleavage of these propeptides serves to activate these proteins. The proteolytic activation of VEGF-C and VEG-F-D is important for receptor binding (a) and is essential for VEG-F-D to promote tumour growth and spread (b). Further, expression of VEG-F-C and VEG-F-D is correlated with poor patient outcome in prevalent human cancers. We previously showed that VEGF-C and VEGF-D activation can be driven by the key fibrinolytic enzyme plasmin (c), but the significance of this for tumour biology in vivo remained unknown. Here we use in vivo tumour models to explore the importance of plasmin-mediated activation of VEGF-D for tumour biology. We show that plasmin is a key enzyme for promoting the metastatic spread of cancer driven by VEGF-D.

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**ISCP/AVBS Session – TREATING PATIENTS WITH CORONARY DISEASE**

**67: NOVEL WAYS TO INHIBIT PLATELETS WITHOUT BLEEDING COMPLICATIONS**

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Platelet inhibition has been proven to prevent cardiovascular events, both in primary as well as secondary prevention. Newer anti-platelet drugs have been shown to be more potent and thereby indeed deliver better protection from thrombotic events. However, with the currently clinically used anti-platelet drugs there seems to be an inherent link between potency and bleeding complications. Indeed, such bleeding complications induced by medication is becoming a major determinant of the morbidity and mortality in patients. However, there are new anti-platelet strategies available that seem to break this link, providing potent anti-thrombotic protection without increased bleeding risk. Amongst those are therapeutic approaches directed against platelet GPVI, platelet alpha6-beta1 and GPIIb/IIa. The latter approach differs from the currently in the clinic used GPIIb/IIa inhibitors in providing inhibition only of the activated GPIIb/IIa receptor and thus of the activated platelet. Furthermore, a whole new group of anti-thrombotic drugs can be designed using clot targeting as a tool to enrich anti-platelet drugs at the developing clot and thereby avoiding high systemic concentrations typically associated with bleeding complications. Clot targeting of CD39, a naturally occurring ADPase, in a new recombinant molecule is such a promising novel approach resulting in platelet inhibition by degrading ADP specifically at the clot without requiring high system concentrations that would cause bleeding problems. Overall, there are several new strategies available that might ultimately allow delivering the much needed anti-thrombotic benefits without causing bleeding complications.
68: OBSERVATIONAL DATA / USE OF STABILISED TREATMENTS

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There is a large evidence base for the management of patients with Acute Coronary Syndromes, this includes the use of reperfusion therapy for ST Elevation MI, invasive strategy for Non ST Elevation MI, Pharmacological Therapy with dual antiplatelet therapy, lipid lowering, beta blockers and ace inhibitors. Despite the evidence base and clear guidelines from AHA / ACC / ESC and Australia many patients miss out on these effective therapies. This has been well demonstrated by recent data from observational registries in Australia & New Zealand ACACIA & SNAPSHOT. Further it is often the patients who potentially might benefit the most that miss out on therapies, particularly invasive management. The use of pharmacological therapy varies across hospitals, individuals and regions and relates to a number of factors including doctor preferences, beliefs about the evidence, e.g. beta blocker, patient preference and cost. If we instituted all our known therapies we would improve on secondary prevention more than some of the newer therapies that have been tested in recent times.

69: NOVEL MANAGEMENT OF STABLE ANGINA PECTORIS

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Chronic stable angina is the most common manifestation of ischaemic heart disease in the developed world and is associated with impaired quality of life and increased mortality. The pathogenesis of stable angina is complex and often, albeit not always, involves flow-limiting epicardial coronary artery stenoses that reduce blood supply to the myocardium. Abnormalities of the coronary microcirculation can also play an important pathogenic role. An imbalance between myocardial oxygen supply and metabolic oxygen demand triggers angina pectoris and represents a major therapeutic target. Rational treatment requires a multi-faceted approach combining lifestyle changes, aggressive management of modifiable coronary artery disease risk factors, pharmacological therapy and myocardial revascularisation, when appropriate. Several new anti-anginal drugs have been introduced to the armoury that might allow more effective symptom control. These novel agents have specific mechanisms of action and fewer side effects compared to conventional drugs. The combined use of traditional and novel treatments is likely to increase the proportion of patients who are managed successfully with medical therapy alone. Importantly, an understanding of the prevailing pathogenic mechanisms leading to myocardial ischaemia in the individual patient can help select appropriate therapeutic strategies and improve clinical outcome.

71: SHEAR STRESS RECRUITS THE TRPV4 ION CHANNEL IN ACETYLCHOLINE-DEPENDENT VASODILATATION

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Background and Aims: Vascular blood flow causes shear stress on the endothelium. The cation channel TRPV4 is implicated in sensing shear stress and subsequent flow-mediated, endothelium-dependent vasodilatation. Intracellular signalling from G-protein coupled receptors, such as muscarinic receptors, activates TRPV4. This study investigated the involvement of TRPV4 in endothelium-dependent vasodilatation in the presence and absence of shear stress.

Methods: Responses to the TRPV4 agonist GSK10167904 (GSK, 10 nM-10 μM) and the muscarinic receptor agonist acetylcholine (ACh, 10 nM-10 μM) were measured in cannulated and pressurised small resistance rat cremaster arterioles which display myogenic tone. Lumenal flow enhanced the dilatation to GSK but not ACh.

Results: ACh and GSK caused concentration-dependent dilatation (pEC50 ACh 6.42 ± 0.22, GSK 7.60±0.23) which in each case was abolished by removal of the endothelium. The GSK-induced dilatation was unaffected by inhibition of nitric oxide synthase and cyclooxygenase with L-NAME (100 μM) and indomethacin (10 μM) but was abolished by the additional inhibition of SKCa and IKCa channels with apamin (1 μM) and TRAM-34 (1 μM). The GSK-induced dilatation was significantly impaired by the TRPV4 antagonist HC067047 (HC, 300 nM; pEC50 6.80±0.12, p<0.05) but responses to ACh were unaffected. When shear stress was generated by flow of 100 μl per min through the lumen there was a significant enhancement of the sensitivity to GSK (flow pEC50 8.52±0.23, p<0.05) but not to ACh (pEC50 6.55±0.14). However, following the addition of shear stress responses to ACh could be significantly inhibited by HC (300nM pEC50 5.95±0.14, p<0.05).

Conclusions: In the rat cremaster arteriole activation of TRPV4 causes endothelium-dependent dilatation that involves opening of SKCa and IKCa but is independent of NO or cyclooxygenase products. Lumenal flow enhanced the dilatation to GSK but not ACh. The application of shear stress incorporates TRPV4 into the signalling mechanisms of endothelium-dependent vasodilatation to ACh. This indicates that shear stress alters the function of TRPV4 in the vascular endothelium. This study shows that there is a specific collection of inter-dependent factors that lead to involvement of TRPV4 in ACh-dependent vasodilatation. We conclude that shear stress enhances TRPV4 mediated vasodilatation and alters the function of TRPV4 in the endothelium. Thus, understanding the effect of shear stress on TRPV4 is a vital component in understanding myogenic regulation.
Background: Endogenous up-regulation of PVN Gai, proteins facilitates sympathoinhibition and normotension during chronic Na\textsuperscript{+} challenge in Sprague-Dawley rats via a renal-nerve dependent mechanism.

Aim: To examine the role(s) of PVN Gai, proteins in Na\textsuperscript{+} homeostasis and MAP regulation during high salt-intake in intact and renal denervated (RDNX) Dahl salt-resistant (DSR) and salt-sensitive (DSS) rats.

Methods: Sham or RDNX DSR and DSS rats receiving a continuous i.c.v. infusion of a scrambled (SCR) or Gai, oligodeoxynucleotide (ODN; 25 µg/day) were fed a 21-day normal 0.4% (NS) or high (8%) NaCl (HS) chow. MAP was recorded continuously by radiotelemetry and Na\textsuperscript{+} homeostasis was assessed via metabolic balance studies. On day 21 plasma norepinephrine (NE), autonomic function and PVN Gai, protein levels were determined (n=6/group).

Results: HS-intake did not alter MAP, suppressed plasma NE (P<0.05), and evoked a site-specific increase in PVN Gai, protein levels in salt-resistant BN and DSR rats (4.8 and 4.5-fold respectively; P<0.05), but not DSS rats. In DSR rats Gai, down-regulation evoked rapid renal nerve-dependent hypertension (MAP [mmHg] Day 4 HS Sham 121±2* vs. RDNX 101±2, Day 21 HS Sham 127±2* vs. RDNX 101±1), sodium retention, and global sympathoexcitation (plasma NE [nmol/L] Sham 87±7* vs. RDNX 37±3, peak ΔMAP to chlorisondamine [mmHg] Sham –68±4* vs. RDNX –37±3). In DSS rats, chronic central Gai, ODN infusion exacerbated salt-induced hypertension (Day 4 HS Sham 148±5* vs. RDNX 133±4, Day 21 HS Sham 175±4* vs. RDNX 145±3), sodium retention, and global sympathoexcitation (plasma NE [nmol/L]: Sham 118±9* vs. RDNX 88±6), peak ΔMAP (mmHg) to chlorisondamine: Sham –76±5* vs. RDNX –55±5) in a renal nerve dependent manner, where * signifies P<0.05 vs. RDNX+ HS.

Conclusion: PVN Gai, protein-gated pathways represent a conserved central molecular pathway mediating the endogenous central sympathoinhibitory renal-nerve dependent mechanisms activated to maintain fluid and electrolyte homeostasis and normotension in salt-resistant phenotypes during high salt challenge. Owing to SNPs in the GNAI2 gene correlating with increased hypertension risk in Italian and Japanese populations, our findings have translational implications for human hypertension.

37: THE DISTRIBUTION OF SOME SINGLE NUCLEOTIDE POLYMORPHISMS OF THE RENIN-ANGIOTENSIN SYSTEM IN INDIGENOUS AUSTRALIANS

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Background: Some single nucleotide polymorphisms (SNPs) in genes can be associated with the alteration in activity of the renin-angiotensin system (RAS) and are increased in prevalence in non-Indigenous patients who have cardiovascular and other chronic diseases. It has also been shown that RAS SNPs can affect the efficacy of drugs that act on the RAS. There is a greatly increased prevalence of hypertension, diabetes (3.4-fold) and chronic kidney disease (10-fold) in Indigenous Australians and these conditions may be associated with the presence of SNPs that alter overall function of the RAS. A high frequency of those RAS SNPs known to be associated with cardiovascular disease in non-Indigenous people might contribute to the increased risk of cardiovascular and renal disease in Indigenous Australians.

Aim: To compare, in Indigenous and non-Indigenous Australians, the frequency of 6 RAS SNPs. These SNPs have been identified in non-Indigenous people as being associated with either increased levels of their products, hypertension, or metabolic disease.

Methods: Genomic DNA previously extracted from whole blood samples collected from an isolated community of Indigenous Australians (n=109) was compared with DNA obtained from the buffy coat of samples from healthy blood donors to the Australian Red Cross Blood Service (n=153). Commercially available real-time polymerase chain reaction (RT-PCR) allelic discrimination assays (PE Applied Biosystems, Foster City, California, USA) were used for the identification of the following gene polymorphisms: AGT G–217A (rs5049), AGT G+174A (rs4762), AGTR1 A+1166C (rs5186), ACE A–240T (rs4291), ACE T–93C (rs4292) and REN T+1142C (rs5706). The study was approved by the elders of a central desert community, and assessed by the University of Newcastle Human Research Ethics Committee and the Hunter New England Health Research Ethics Committee.

Results: Two SNPs in the ACE gene, A–240T and T–93C, were significantly more frequent in the Indigenous cohort than in the non-Indigenous cohort (P<0.0001). SNP AGT G+174A was also found to be more frequent (P<0.0001). SNPs AGT G–217A and AGTR1 A+1166C were both significantly less frequent in the Indigenous cohort (P<0.0001). There was no difference in the frequency of the T+1142C SNP of the renin gene.

Conclusions: Our findings show that the frequency of RAS polymorphisms is very different in an Indigenous Australian community compared with non-Indigenous Australians. The potential contribution of these differences to the risk of cardiovascular and renal disease in Indigenous Australians merits further investigation.
74: FOXO3 GENOTYPE INCREASES LIFESPAN BY A MAJOR EFFECT ON CARDIOVASCULAR MORTALITY

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Background: The strongest, most consistently replicated genetic influences on human lifespan worldwide involve specific alleles of the ApoE and FoxO3 genes. For FOXO3, the G allele of SNP rs2802292 exhibits the most robust association.

Aim: To determine what particular cause(s) of death the longevity-associated FOXO3 genotype protects against.

Methods: Cause-specific mortality over 9 years was determined in 3,584 elderly American men of Japanese ancestry from the Kuakini Hawai lifespan Study. The latter is an extensively characterized, long-studied, robust cohort drawn from the Honolulu Heart Program, which recruited subjects in 1965–1968. We used a Cox proportional hazards model to compute relative risk of mortality for coronary heart disease (CHD), cancer, stroke, other circulatory diseases, and remaining causes. We created multivariate models that included other major risk factors for all-cause and cause-specific mortality. A replication study was performed using the Health Aging and Body Composition cohort of 3,075 community-dwelling white and black Americans from field centers in Pittsburg and Memphis.

Results: The survival curve, age standardized at 78 years (mean subject age at baseline), for carriers and non-carriers of the longevity-associated G allele showed that G allele carriers had longer survival (P=0.016) during follow-up over 9 years (1,272 total deaths). Over the 9 years of follow-up, the 1,683 carriers of the G allele exhibited risk reductions of 16% (hazard ratio [HR] 0.88; 95% CI 0.81–0.95; P=0.0019) for all-cause mortality (1,272 deaths), 38% (HR 0.62; 95% CI 0.48–0.80; P=0.0019) for CHD mortality (257 deaths) and 44% (HR 0.56; 95% CI 0.35–0.90; P=0.018) for infectious diseases (73 deaths). G allele carriage was not associated with other causes of death. In the replication study, protection against CHD was 24% (HR 0.76; 95% CI 0.58–0.99; P=0.039) in whites and 29% (HR 0.71; 95% CI 0.40–1.26; P=0.24) in blacks. For all racial groups combined, protection against CHD conferred by G allele carriage was 31% (HR 0.69; 95% CI 0.50–0.82; P=0.00002).

Conclusion: The G allele of the FOXO3 SNP rs2802292 is associated with a major effect on mortality, principally through reduction in CHD risk, independent of known major risk factors. The findings support research on FoxO3, its gene and downstream effectors as targets for therapeutic interventions in healthy aging.

75: MicroRNAs MEDIATE THE PROTECTIVE EFFECT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION (ACEI) ON THE HEART IN RAT MODEL OF ACUTE KIDNEY INJURY

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Introduction: Cardiovascular disease is the major cause of death in patients with kidney disease. Cardiac fibrosis and hypertrophy are common in patients with kidney injury disease, and can be partially attenuated using blockers of the renin angiotensin system (RAS). MicroRNAs are a class of small noncoding RNAs that modulate gene expression at the post-transcription level. They play an important role in regulating cell death pathways and cardiac hypertrophy, but it is not known whether cardiac microRNAs contribute to cardio-renal cross talk. microRNA-1 and microRNA-133 are associated with cardiac fibrosis and apoptosis after myocardial infarction, whilst microRNA-212/132 is associated with cardiac hypertrophy.

Aim: Using a rat model we investigated whether acute kidney injury leads to changes in levels of cardiac microRNAs -1, -133, -212 and -132 and their mRNA targets. We also tested the effect of treatment with the angiotensin converting enzyme inhibitor (ACEi) ramipril on these cardiac microRNAs.

Methods: Heart tissues were collected from rats 10 days after subtotal nephrectomy (STNx) surgery, where one kidney was removed and the other was partially ligated (n=9), from sham animals (n=9), and from STNx rats treated with ramipril (n=9). RNA was extracted from the left ventricle and quantitative real-time PCR was used to measure microRNA and their target transcript levels.

Results: In rats with renal injury, there was a significant increase in left ventricular hypertrophy (P<0.05 vs sham). There was also a significant increase in cardiac microRNA-212 (2-fold; P<0.05 vs Sham) and microRNA-132 (3-fold; P<0.05 vs Sham). Ramipril treatment in STNx rats attenuated the increase in microRNA-212 and caused a significant increase in microRNA-133 (P<0.001 vs Sham; P<0.001 vs STNx+Veh) and microRNA-1 (P<0.01 vs Sham; P<0.01 vs STNx+Veh). Renal injury induced left ventricular hypertrophy was attenuated in ramipril treated STNx rats (P<0.001 vs STNx+Veh). We also found alteration in mRNA levels of caspase 9, fibronectin 1, collagen 1A1 and forkhead box O3, all known for their involvement in the regulation of apoptosis, fibrosis and hypertrophy in cardiac cells, whilst being targets for the above microRNAs.

Conclusions: The involvement of microRNAs in cardiac pathologies associated with kidney injury is a novel finding of our study. Our finding suggests an involvement of microRNA-1, microRNA-133 and microRNA-212/132 in the...
76: GENETIC DEFICIENCY OR PHARMACOLOGICAL DEPLETION OF B CELLS PREVENTS ANGIOTENSIN II-INDUCED HYPERTENSION IN MICE


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Background: B cells are the antibody-producing cells of the mammalian immune system. While there is evidence that serum antibody levels are raised in patients with hypertension, no studies have examined whether a cause-effect relationship exists between B cell activation and hypertension.

Aims: To determine whether hypertension in mice is associated with B cell activation and to assess whether B cell deficiency affords protection against hypertension.

Methods: Wild-type mice and B cell activating factor-receptor deficient (BAFF-R–/–) mice (deficient in B cells) were infused with Ang II (0.7 mg/kg/d, s.c.) or saline for 28 days. Some wild-type mice were also treated with a B cell depleting anti-CD20 antibody, beginning 3 weeks before Ang II infusion. Serum antibody levels were quantified using a Luminex Immunoassay and aortic IgG2b antibody deposits were examined via immunohistochemistry. B cell numbers in the spleen, para-aortic lymph nodes, kidney and thoracic aorta were measured by flow cytometry, while tail-cuff plethysmography and radiotelemetry were used to measure blood pressure (BP).

Results: Ang II infusion in wild-type mice was associated with an approximate doubling of serum levels of IgG2b and IgG3 (P≤0.10), and evidence of an increase in aortic deposits of IgG2b in the adventitia and perivascular adipose tissue. Although Ang II treatment was not associated with increased B cell numbers in any organs analysed, the proportion of activated B cells (i.e., CD86+ B cells) was higher in spleens and para-aortic lymph nodes of Ang II-treated mice (P<0.05). To determine whether B cell activation and increased antibody production might contribute to hypertension, pressor responses to chronic Ang II infusion were compared in wild-type and BAFF-R–/– mice. Ang II increased systolic BP in wild-type mice by ~50 mm Hg (P<0.05). In BAFF-R–/– mice, the pressor response to Ang II was attenuated by ~25% (P<0.05). Depletion of B cells in wild-type mice with an anti-CD20 antibody also blunted the pressor response to Ang II by ~50% (P<0.05). Importantly, adoptive transfer of B cells into BAFF-R–/– mice restored the BP response to Ang II to levels seen in wild-type mice (P<0.05).

Conclusions: The present study provides the first direct evidence that B cell activation is crucial to the development of Ang II-dependent hypertension. Although the mechanisms by which B cells contribute to hypertension remain to be explored, these findings highlight activated B cells and possibly antibodies as potential targets for future antihypertensive therapies.

77: A NOVEL INSERTIONAL SOMATIC KCNJ5 MUTATION IN AN AUSTRALIAN PATIENT WITH AN ALDOSTERONE-PRODUCING ADENOMA

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Background: Primary aldosteronism (PA), in which there is excessive and autonomous production of aldosterone by one or both adrenal glands, accounts for around 5–10% of hypertension. PA may be unilateral (usually due to aldosterone-producing adenoma [APA] and correctable by unilateral adrenalectomy) or bilateral (usually treated medically with agents that antagonize aldosterone action). Recently, somatic mutations in the gene KCNJ5 (encoding a potassium channel) have been detected in about 40% of surgically removed APAs.

Aim: To screen for additional somatic mutations in KCNJ5 in a cohort of APAs removed from 87 Australian patients.

Methods: The full-length coding sequence and flanking regions of KCNJ5 in APA and adjacent cortex were resequenced. Functional changes caused by a novel mutation were studied in vitro by expressing wild-type (WT) or the mutant KCNJ5 channel in Xenopus oocytes (to examine electrophysiological effects) and by transflecting empty GFP vector or the GFP-tagged mutant channel in human adrenocortical carcinoma (H295R) cells (to assess aldosterone release).

Results: KCNJ5 mutations were detected in 37 APAs, and included the previously reported E145Q (n=3), G151R (n=20) and L168R (n=13) mutations plus a novel 12-bp mutation, c.414-425dupCGCTTTCCTGTT (A139_F142dup) that duplicates the AFLP sequence just upstream of the selectivity filter. No mutations were found in adjacent cortices. On expression in Xenopus oocytes, the A139_F142dup mutation reduced the resting membrane potential and showed a substantial loss of channel selectivity for potassium (K/Na permeability ratio 31 in WT KCNJ5 channels versus 7 in the A139_F142dup mutant). When transfected into H295R cells, A139_F142dup, increased basal aldosterone release 2.3-fold compared to WT. This was not increased further by incubation with Angiotensin II. Clinically, the 54-year-old male from whom the mutation-bearing APA was removed had relatively severe PA with resistant hypertension, markedly elevated aldosterone/renin ratio (aldosterone 490 pmol/L and renin 2 mU/L: ratio 296) and an 11 mm left adrenal tumour on CT with lateralization to that side on adrenal venous sampling.

Conclusions: Resequencing of a large Australian cohort of patients with APA further confirmed the major role of KCNJ5 somatic mutations in APA. The novel duplication mutation we report here has similar functional effects to
the other mutations affecting the selectivity filter of the KCNJ5 channel with reduced membrane polarization, reduced selectivity to K and increased aldosterone release.

AAS Session - OXIDATIVE STRESS

78: THE EFFECT OF DIETARY POLYPHENOL ‘ANTIOXIDANTS’ ON VASCULAR FUNCTION AND BLOOD PRESSURE

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Population studies suggest cardiovascular health benefits of consuming fruits and vegetables which may be due in part to their high content of polyphenolic compounds. While many plant derived polyphenols, such as flavonoids, display potent antioxidant activity in vitro, the in vivo effects of these compounds is more likely the result of specific actions on key enzymes in the vasculature rather than total antioxidant effects. Certain dietary flavonoids can acutely augment nitric oxide production and reduce endothelin-1 in human volunteers. Quercetin derived from apples can also increase nitric oxide production and improve endothelial function in healthy subjects. We have also shown in a long term (6 months) randomised controlled trial that black tea polyphenols can significantly reduce blood pressure in subjects with high-normal BP.

Incorporation of quercetin into the diet of the apoE knockout mice can reduce lesion formation significantly by a combination of anti-inflammatory, antioxidant and increased expression of heme oxygenase-1 (Hmox-1) in aortic vessels. In mouse aortic rings, quercetin protects vessels from oxidant (HOCl)–induced endothelial dysfunction ex vivo. HOCl is a physiological oxidant generated in atherosclerotic lesions. We conclude that certain dietary polyphenols may be beneficial for cardiovascular health and is related to their sensitivity to oxidation rather than their ‘antioxidant’ activity.

79: BILIRUBIN AND ATHEROSCLEROSIS: COULD PREVENTION OF OXIDATIVE STRESS CONTRIBUTE TO PROTECTION?

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Bilirubin is an endogenous antioxidant and is continuously produced as a by-product of haem catabolism. Many in vitro investigations demonstrate that bilirubin prevents lipid and protein oxidation, providing evidence to suggest bilirubin can compete with macromolecules for oxidation. Despite these promising observations, the relevance of bilirubin in protecting from oxidative modification and potentially, atherosclerosis, in vivo remains elusive.

In order to explore one potential mechanism whereby bilirubin might protect from atherosclerosis, we have investigated whether exogenously or endogenously elevated bilirubin prevents protein and lipid oxidation. We adopt a translational approach of in vitro, and ex vivo experimentation, utilising blood samples from animal (Gunn rat) and human (Gilbert’s Syndrome) models of hyperbilirubinemia to demonstrate the potential of bilirubin to prevent oxidation, which may partly explain CVD protection in a substantial proportion of the human population.

80: NOVEL ROLES OF HEME OXYGENASE-1 IN VASCULAR PROTECTION

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We previously observed that certain antioxidants protect against atherosclerotic disease via induction of the heme-degrading enzyme heme oxygenase-1 (Hmox1), although the mechanisms involved remain largely unknown. We now show that in the case of ischemia-mediated neovascularization, Hmox1 regulates hypoxia-mediated energy reprogramming via stabilization of hypoxia-inducible factor 1alpha, likely via modulation of mitochondrial reactive oxygen species. In the case of the hyperproliferative response of vascular smooth muscle cells associated with intimal hyperplasia, protection depends on the cleavage of the transmembrane C-terminal 23 amino acids of Hmox1. Such stress-induced truncation of Hmox1 is associated with the translocation of the enzyme from the endoplasmic reticulum to the nucleus. Truncated Hmox1 retains enzymatic activity that appears to be required for the protective activity of the enzyme. Together, our results suggest that Hmox1-mediated metabolism of heme in the nucleus may serve a biological purpose, and that Hmox1 is upstream of hypoxia-inducible factor 1.
Background and Aims: A critical early event in the pathogenesis of cardiovascular diseases such as atherosclerosis is vascular inflammation leading to endothelial dysfunction (ED). Reactive oxygen species and inflammation are intrinsically linked and declining antioxidant defense is implicated in ED. We have previously shown that Glutathione peroxidase-1 (GPx1) is a crucial antioxidant enzyme in the protection against diabetes-associated atherosclerosis. In this study we aimed to investigate mechanisms by which lack of GPx1 affects pro-inflammatory mediators in primary aortic endothelial cells (PAECs) isolated from GPx1 knockout (GPx1KO) mice.

Methods: Wild-type and GPx1-deficient PAECs were isolated from 6-week old mice and cultured for 6-8 passages. Pro-inflammatory TNF-α (20ng/ml) was added to cells for 15, 30 and 60 mins. Cells were harvested and phosphorylation of MAPK proteins, ERK1/2, JNK and p38 and the inhibitory subunit IκB of NF-κB were examined by Western blot (WB). Cellular adhesion molecule VCAM-1 protein levels were assessed after 4h of TNF-α treatment. Additionally, the effect of 1h pre-treatment with the GPx1 mimetic ebselen on MAPK proteins and VCAM-1 was assessed after TNF-α treatment. p-AKT, the upstream regulator of eNOS, was also assessed by WB. Aortas were isolated from WT and GPx1KO mice and an ex-vivo dynamic flow assay performed to investigate adhesion of leukocytes to TNF-α activated vascular endothelium in real time. Aortas were also pre-treated with ebselen for 2hr prior to ex-vivo analysis.

Results: Lack of GPx1 prolonged TNF-α induced phosphorylation of JNK, p38 and ERK, and prolonging the degradation of IκB. Expression of VCAM-1 was significantly increased in GPx1KO PAECs. Treatment with ebselen abrogated the prolongation of MAPK signaling and lessened VCAM-1 levels. p-AKT was significantly lower in GPx1KO PAECs. Dynamic flow assays showed significantly increased adhesion of fluorescent labelled inflammatory cells to GPx1KO aortas. This effect was significantly reduced by ebselen (P<0.001 vs TNF-α treated GPx1KO aorta).

Conclusions: Our results suggest that GPx1 plays a critical role in regulating pro-inflammatory pathways, including MAPK and NF-κB, and down-stream mediators such as VCAM-1, in vascular endothelial cells. Lack of GPx1, via effects on p-AKT also affects signalling to eNOS. We speculate based on these results that declining antioxidant defenses as seen in diabetes, by failing to regulate these pro-inflammatory pathways, facilitates an inflammatory and activated endothelium leading to ED and atherogenesis.

82: NEOPTERIN IS A DIRECT MARKER OF Γ-INTERFERON ACTIVATION OF MACROPHAGES AND OXIDATIVE STRESS WITHIN HUMAN ATHEROSCLEROTIC PLAQUE

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Background: The severity of cardiovascular disease is strongly correlated with the level of neopterin in patients plasma. Neopterin is the oxidation product of 7,8-dihydroneopterin, a pterin compound generated and released by γ-interferon activated macrophages. 7,8-dihydroneopterin is a potent antioxidant and down-regulator of macrophage scavenger receptor CD36. This compound is therefore both a marker and effector of the disease process. The source of the plasma neopterin has always been inferred but never proven to be from the macrophages within the atherosclerotic plaques. We have examined both plasma and plaque of cardiovascular disease patients to measure the plaque capacity to generate 7,8-dihydroneopterin and neopterin.

Methods: The plasma levels of neopterin and total neopterin (7,8-dihydroneopterin and neopterin combined) were measured in 27 patients undergoing carotid endarterectomy surgery and compared to age matched health controls. Selected surgically removed carotid plaques were sliced into 3mm thick rings and cultured in serum supplemented RPMI1640 media for up to 96 hours during which time they were stimulated with γ-interferon. The pterin levels in both the plaque culture media and plasma were measured by SCX-HPLC with fluorescence detection.

Results: The addition of γ-interferon caused a large and significant rise in both neopterin and total neopterin for all segments of cultured plaque. The increase in 7,8-dihydroneopterin was between 0.7 and 3.7 nmol/g of tissue. There was a significant difference in the extent of this activation between plaque segments and the levels of 7,8-dihydroneopterin to neopterin oxidation. These variations demonstrate the highly dynamic state within the plaque. Analysis of 7,8-dihydroneopterin solution incubated with various oxidants showed that both HOCI and myoglobin readily oxidised 7,8-dihydroneopterin to neopterin. Analysis of the patients plasma showed the levels of total neopterin and neopterin were elevated 25% higher than the controls. This showed that not only were the patients plaques generating more 7,8-dihydroneopterin, a greater amount was also being oxidised to neopterin.

Conclusion: Our study demonstrates for the first time that atherosclerotic plaques do generate 7,8-dihydroneopterin and the measurement of both 7,8-dihydroneopterin and neopterin may provide a measure of the dynamic state of patients atherosclerotic plaques.

83: A CRITICAL LOOK AT NOVEL APPROACHES TO LDL LOWERING

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Elevated LDL cholesterol increases the risk of coronary disease. The association has mechanistic plausibility because of human genetic data, such as those from patients with familial hypercholesterolemia and from Mendelian randomisation studies, and from animal studies manipulating the LDL receptor. It is strongly supported by clinical studies with statins which have shown important reductions in morbidity and mortality in most (but not all) patient groups roughly in proportion to the degree of LDL lowering. New therapies to lower LDL are being actively investigated because of the large proportion of patients on a statin who do not achieve “target” LDL cholesterol and because many patients are intolerant of statins. These include the PCSK9 inhibitors, the apoB antisense oligonucleotides, microsomal transfer protein inhibitors and cholesterol absorption inhibitor ezetimibe. We will explore how these agents may differ from and/or complement statins and ask whether all drugs that lower LDL cholesterol will have similar benefits. The potential importance of the route by which LDL is lowered – in particular hepatic clearance via the LDL receptor- will be considered.

84: IS THERE A FUTURE FOR CETP INHIBITORS?

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There is great current interest in the potential of cholesteryl ester transfer protein (CETP) inhibition as a strategy to reduce the risk of developing atherosclerotic cardiovascular disease (ASCVD). There is also great confusion. On one hand, inhibition of CETP has been shown in humans to reduce the concentration of cholesterol in atherogenic lipoproteins and in rabbits to inhibit development of atherosclerosis. On the other hand, two large clinical outcome trials with CETP inhibitors failed to show a reduction in ASCVD.

However, evidence that the failure of these trials may have been due to adverse off-target drug effects unrelated to CETP in one trial and to problems with the population studied in the other, has provided equipoise in a decision to proceed with further testing of the hypothesis that CETP inhibition is anti-atherogenic. Two large clinical outcome trials with CETP inhibitors that do not have adverse off-target effects are currently underway. It is essential that these ongoing trials proceed to completion as planned in order to provide an answer to one of the more important unanswered questions related to strategies for reducing the risk of ASCVD.

85: HDL-TARGETED THERAPIES: PROGRESS, FAILURES AND FUTURE

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Baker IDI Heart and Diabetes Institute

Since the discovery in the 1970s that plasma levels of high-density lipoprotein cholesterol (HDL-C) are inversely associated with cardiovascular outcome, it has been postulated that HDL is anti-atherogenic and that increasing HDL-C levels is a promising therapeutic strategy. However, the recent failure of three orally active, HDL-C-raising agents has introduced considerable controversy, prompting the question of whether increasing the cholesterol cargo of HDL in a non-selective manner is an effective pharmacological approach for the translation of its atheroprotective and vasculoprotective activities. The interrelationships between HDL-C concentration, HDL particle number and levels of diverse HDL particle subpopulations of defined composition are complex, as are their relationships with reverse cholesterol transport and other anti-atherogenic functions. Such complexity highlights the incompleteness of our understanding of the biology of HDL particles. This presentation will discuss the HDL hypothesis in molecular and mechanistic terms, focusing on emerging strategies for HDL therapies.


86: REGRESSION OF CORONARY ATHEROSCLEROSIS IN RESPONSE TO INFUSION OF HIGH-DENSITY LIPOPROTEIN MIMETIC AGENT CER-001 IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background and Aim: Several lines of evidence have stimulated considerable interest towards high-density lipoprotein (HDL)-directed therapy due to its anti-atherosclerotic properties such as cholesterol efflux, anti-inflammatory and anti-oxidative effects. The infusion of HDL represents a novel approach to modulate atherosclerosis and potentially reduce cardiovascular events. While recent clinical trials using intravascular ultrasound (IVUS) have suggested a potential benefit of HDL infusion on plaque progression in small study populations, this therapy has not yet...
been definitively established due to lack of significance versus placebo. The CHI-SQUARE (Can Hdl Infusions Significantly QUicken Atherosclerosis REgression) study aimed to evaluate the efficacy of infusion of the pre-beta HDL mimetic agent CER-001 on atherosclerotic plaque in patients with acute coronary syndrome (ACS).

**Methods:** The CHI-SQUARE study was a prospective, double-blinded, randomized multi-center trial. Serial IVUS imaging was conducted to compare plaque volume in 507 ACS patients treated with 6 weekly infusions of placebo, or CER-001 at 3, 6 or 12 mg/kg. Of these, 369 patients with evaluable paired IVUS images were analysed in our core lab. Percent atheroma volume (PAV) and total atheroma volume (TAV) were measured. Serial changes in these measures were compared between placebo and each dose level of CER-001.

**Results:** There were no significant differences with regard to baseline PAV and TAV among 4 groups ($p=0.41$ and 0.28, respectively). On serial evaluation in the modified per protocol population ($n=295$), infusion of 3 mg/kg CER-001 was associated with a significant regression of PAV (change in PAV = -0.69%, $p=0.02$ vs. baseline; placebo group: -0.13%, $p=0.65$ vs. baseline, $p=0.05$ between groups). No significant differences were observed in plaque progression rate in 6 or 12 mg/kg CER-001 groups (change in PAV: -0.32 and +0.34%, $p=0.24$ and 0.25 vs. baseline, $p=0.27$ and 0.47 vs. placebo group). With regard to TAV, patients treated with 3 mg/kg CER-001 exhibited the greatest reduction in TAV (-3.21, -5.64, -3.03 and -1.75 mm3 in placebo, 3, 6 and 12 mg/kg CER-001, $p<0.001$ vs. baseline, $p=0.04$ between placebo vs. 3 mg/kg).

**Conclusion:** Regression of coronary atherosclerosis at the lowest dose of CER-001 was observed in patients with ACS after 6 weeks on top of LDL-C lowering therapy. This inverse dose-response has precedent in prior experiences with HDL mimetics, including apoA-Imilano. The clinical implications of this finding require further investigation.

**HBPRCA Session - TREATMENT OF HYPERTENSION**

**87: ANATOMICAL AND PHYSIOLOGICAL PROFILING TO IDENTIFY APPROPRIATE THERAPY FOR TREATMENT RESISTANT HYPERTENSION**

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**Background:** In patients with treatment resistant hypertension (TRH) exclusion of secondary causes with imaging and endocrine testing is recommended. We proposed that a single magnetic resonance imaging (MRI) visit could provide all the anatomical information required in routine evaluation of patients with hypertension (HTN).

**Aim:** To explore the utility of advanced imaging and physiological testing in targeting therapy in patients with TRH.

**Methods:** Patients attending a specialist hypertension clinic with early onset (<40 years), multi-drug intolerant HTN or TRH underwent a Hypertension Protocol MRI scan for assessment of end organ damage and secondary causes. This included imaging of the renal arteries, adrenals, aorta and heart as well as cerebral vessels. This was performed in conjunction with standard endocrine testing. Data were presented as mean±SD. Selected patients with TRH underwent autonomic profiling with measurement of muscle sympathetic nerve activity, baroreceptor sensitivity and carotid body activity.

**Results:** 109 patients (61 male), aged 53±14 years, had an office systolic/diastolic blood pressure of 174±28 / 99±16 mmHg on 3.4±2.0 antihypertensive medications. MRI identified 10 myocardial infarcts, 3 hypertrophic cardiomyopathies, 1 multinodular goitre, 5 renal artery stenoses, 5 adrenal masses and 6 cerebral microaneurysms. 26% had ≥1 accessory renal artery and 29% had ≥1 hypoplastic vertebral artery. 80% of patients had left ventricular hypertrophy by left ventricular mass index (94.5±27.6 g/m²). MRI identified 10 myocardial infarcts, 3 hypertrophic cardiomyopathies, 1 multinodular goitre, 5 renal artery stenoses, 5 adrenal masses and 6 cerebral microaneurysms. 26% had ≥1 accessory renal artery and 29% had ≥1 hypoplastic vertebral artery. 80% of patients had left ventricular hypertrophy by left ventricular mass index (94.5±27.6 g/m²). No significant differences were observed in plaque progression rate in 6 or 12 mg/kg CER-001 groups (change in PAV: -0.32 and +0.34%, $p=0.24$ and 0.25 vs. baseline, $p=0.27$ and 0.47 vs. placebo group). With regard to TAV, patients treated with 3 mg/kg CER-001 exhibited the greatest reduction in TAV (-3.21, -5.64, -3.03 and -1.75 mm3 in placebo, 3, 6 and 12 mg/kg CER-001, $p<0.001$ vs. baseline, $p=0.04$ between placebo vs. 3 mg/kg).

**Conclusion:** MRI is a safe and effective method of screening for secondary causes of HTN and assessment of end organ damage. It could replace the combination of echocardiography, renal ultrasound and CT imaging. MRI also provides additional information for patients being considered for renal denervation and, along with advanced physiological assessment, could better target pharmacological and interventional therapies for patients with TRH.

**88: COST-EFFECTIVENESS OF ANGIOTENSIN-CONVERTING ENZYME INHIBITOR-BASED COMPARED TO THIAZIDE DIURETIC-BASED TREATMENT IN AN ELDERLY HYPERTENSIVE POPULATION CONSIDERING DIABETES AS A MAJOR COMORBIDITY**

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**Background:** Cost-effectiveness is an important consideration in choice of type of anti-hypertensive medication.

**Aim:** To examine the cost-effectiveness of angiotensin-converting enzyme inhibitor-based (ACEI) compared to thiazide diuretic-based treatment for hypertension in elderly Australian considering diabetes as a co-morbidity.

**Methods:** We used a cost-utility analysis to estimate the incremental cost-effectiveness ratio (ICER) per quality-adjusted life-year (QALY) gained. Data were used from the Second Australian National Blood Pressure Study, a randomized clinical trial comparing diuretic-based versus ACEI-based treatment in 6,083 elderly (age ≥ 65 years) hypertensive...
The identification of vascular progenitor cells in adult peripheral blood has significant clinical implications for the treatment of the biggest killers in the world, cancer and cardiovascular disease. We recently described and characterized a human umbilical cord blood (UCB) derived CD133+ population of non-adherent endothelial forming cells (naEFCs) which generated perfused vasculature in vivo. Using these cells we have identified novel biomarkers which can be used to isolate naEFCs and have provided new targets for the control of vascular function. Briefly, gene expression analysis of naEFCs compared to donor matched human umbilical vein endothelial cells (HUVEC) revealed that a cell surface adhesion molecule, de-identified here as Bm1, was highly expressed by the naEFC population. Furthermore, flow cytometric analysis confirmed the surface expression of Bm1 on naEFCs but not on other circulating blood lineages, eg CD4+ T cells, CD14+ monocytes, CD16+ natural killer cells, and CD19+ B cells. Functionally, Bm1+ naEFCs are pro-angiogenic as demonstrated by increased tube formation when co-cultured with HUVEC in the Matrigel ring assay and (iii) reduced vessel-like formation in vivo using a Matrigel plug assay. Taken together, this study has identified Bm1 as a new biomarker for naEFCs and implicated this adhesion molecule in the regulation of vascular function both in vitro and in vivo.

AVBS Session - AWARDS SESSION

89: BM1, A NOVEL BIOMARKER AND REGULATOR OF ENDOTHELIAL FORMING CELLS

Parham KA, Ebert LM, Tan LY, Cockshell MP, Lopez AF, Shackleton M, Bonder CS
Centre for Cancer Biology, SA Pathology and University of South Australia, Adelaide, SA, Australia; ‘Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

The identification of vascular progenitor cells in adult peripheral blood has significant clinical implications for the treatment of the biggest killers in the world, cancer and cardiovascular disease. We recently described and characterized a human umbilical cord blood (UCB) derived CD133+ population of non-adherent endothelial forming cells (naEFCs) which generated perfused vasculature in vivo. Using these cells we have identified novel biomarkers which can be used to isolate naEFCs and have provided new targets for the control of vascular function. Briefly, gene expression analysis of naEFCs compared to donor matched human umbilical vein endothelial cells (HUVEC) revealed that a cell surface adhesion molecule, de-identified here as Bm1, was highly expressed by the naEFC population. Furthermore, flow cytometric analysis confirmed the surface expression of Bm1 on naEFCs but not on other circulating blood lineages, eg CD4+ T cells, CD14+ monocytes, CD16+ natural killer cells, and CD19+ B cells. Functionally, Bm1+ naEFCs are pro-angiogenic as demonstrated by increased tube formation when co-cultured with HUVEC in the Matrigel 3-dimensional matrix. Generation of a Bm1-/- mouse has further addressed a role for Bm1 in vascular development. For example, Bm1-/- mice exhibit (i) reduced bone-marrow derived EPC colony formation, (ii) impaired ex vivo vascular sprouting in an aortic ring assay and (iii) reduced vessel-like formation in vivo using a Matrigel plug assay. Taken together, this study has identified Bm1 as a new biomarker for naEFCs and implicated this adhesion molecule in the regulation of vascular function both in vitro and in vivo. Future studies will reveal whether it can be manipulated to combat vascular disease.

90: OPPOSING EFFECTS OF NOX ISOFORMS IN DIABETES ASSOCIATED ATHEROSCLEROSIS

Gray SP, Di Marco E, Chew PFY, Kennedy K, Cooper ME, Wingler K, Schmidt HW, Jandeleit-Dahm KA
Diabetic Complications Division, Baker IDI Heart & Diabetes Research Institute, Melbourne, Australia; “Department of Pharmacology – Vascular Drug Discovery Group, Faculty of Medicine, Health & Life Science, University of Maastricht, The Netherlands

Background: Individuals diagnosed with diabetes have accelerated development of atherosclerosis; however the mechanisms are poorly understood. Oxidative stress appears to play a significant role, specifically NADPH oxidase (Nox)-derived ROS, which are upregulated in activity by glucose. There are three principal isoforms of Nox in the vasculature, Nox1, 2 and 4, which have differing expression and activity profiles within the vasculature.

Aims: To delineate the role of Nox-derived oxidative stress in the development of diabetes-related atherosclerosis. We used Nox isoform specific-ApoE-/- double knockout (dKO) mice, Nox1-/-yApoE-/- and Nox4-/-yApoE-/- mice.

Methods: Mice were rendered diabetic by streptozotocin (55mg/kg/day for 5 days), with non-diabetic wildtype mice serving as controls. Animals were diabetic for a duration of 20 weeks at which point aortas were removed and cleaned for quantification of atherosclerotic plaque area and immunohistochemical analysis, or frozen for RT-PCR analysis.

Results: After 20 weeks of diabetes, all wildtype mice had a significant elevation in atherosclerosis compared to non-diabetic counterparts. Deletion of the Nox1 isoform in diabetes resulted in a significant (50%) reduction in atherosclerosis development compared to Nox1+/-yApoE-/- diabetic mice. In contrast, deletion of the Nox4 isoform in diabetes resulted in a 65% increase in atherosclerosis development compared to Nox4+/-yApoE-/- diabetic mice. Aortic RT-PCR demonstrated a significant reduction in the gene expression of markers for oxidative stress, inflammation (MCP-1, IL1β, TNFα) and fibrosis (Collagen I) in Nox1-/-yApoE-/- diabetic mice, which were significantly elevated in diabetic Nox4-/-yApoE-/- diabetic mice. Immunohistochemistry analysis of the vascular wall identified a significant decrease in pro-oxidant markers and macrophage infiltration in the diabetic Nox1-/-yApoE-/- compared to the Nox1+/-yApoE-/- diabetic mice, which were significantly elevated in diabetic Nox4-/-yApoE-/- mice compared to Nox4+/-yApoE-/- diabetic mice.

Conclusion: These data demonstrate opposing effects of two Nox isoforms in diabetes associated atherosclerosis, Nox1 playing a pathological role to promote a pro-inflammatory and pro-fibrotic environment promoting
atherosclerosis development, where in turn Nox4 derived ROS plays a vasculo-protective role in atherosclerosis development.

91: GLYCOL-SPLIT HEPARIN AS A NOVEL THERAPEUTIC FOR TARGETING MYELOPEROXIDASE-INDUCED ENDOTHELIAL DYSFUNCTION

Chan E, Dang L, Freeman C*, Thai T, Rees MD, Glaros EN, Parish CR*, Thomas SR*
Centre for Vascular Research, UNSW Australia, *John Curtin School of Medical Research, ANU

Background: Myeloperoxidase (MPO) is recognised as a mediator of endothelial dysfunction during cardiovascular disease. Upon release by activated leukocytes, MPO binds to endothelial cells in a heparan sulfate (HS)-dependent manner that is required for the transcytosis and deposition of MPO into the sub-endothelial space. Here MPO catalyses oxidative reactions that impairs endothelium-dependent vasorelaxation by reducing nitric oxide bioavailability. Recent clinical studies support that heparin infusion improves endothelial function in cardiovascular disease patients by mobilising MPO sequestered within the endothelium of diseased arteries. However, long term treatment with heparin is confounded by its anti-coagulant and thrombocytopenic properties. Therefore, recent interest has focussed on the development of chemically modified heparins with reduced side-effects yet capable of preventing deleterious HS-protein interactions.

Aim: To discover modified heparins that target MPO sequestered within the vascular endothelium and improve endothelial function.

Methods and results: ELISA-based screens identified several classes of modified heparins with low anti-coagulant activity, including glycol-split heparin, which efficiently inhibited MPO binding to immobilised HS. Ex vivo studies with isolated aorta showed that glycol-split heparin efficiently removed MPO sequestered within the endothelium and this effectively preserved endothelium-dependent vasorelaxation. To test the protective actions of glycol-split heparin in vivo we employed an MPO-dependent, acute inflammatory model of endothelial dysfunction. In this model, administration of LPS to C57BL/6 mice induced the accumulation of MPO within the arterial endothelium that correlated with increased vascular oxidative stress and impaired endothelium-dependent vasorelaxation. These changes were abrogated in MPO gene knockout mice. Administration of glycol-split heparin inhibited LPS-induced endothelial dysfunction, which correlated with reduced levels of endothelial-sequestered MPO and vascular oxidative stress.

Conclusion: While MPO is implicated in the impairment of endothelium-dependent vasorelaxation, glycol-split heparin represents a promising strategy to remove MPO sequestered within the endothelium and preserve endothelial function during inflammatory cardiovascular disease.
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Heart Failure
Heart failure is often described as the neglected complication of diabetes. Imaging, particularly echocardiography, plays a central role in the management of this complication. For the purposes of this presentation, heart failure will be divided into overt heart failure with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF), and subclinical (stage B) heart failure.

**HFrEF** - The distinction between HFrEF and HFpEF is based upon standard imaging approaches for the calculation of ejection fraction. The accuracy of these techniques for measuring ejection fraction is variable, but this variation is probably less than the heterogeneity of definitions of HFpEF in the large trials! In addition to the measurement of EF, LV volumes, RV function, and diastolic measures including left atrial volume are all prognostically important. Much effort has been directed towards the recognition of mechanical dyssynchrony, but this appears to have limited value.

**HFpEF** - Part of the problem with the lack of a specific treatment for HFpEF is that the accuracy of this diagnosis is poor (ie. many patients so labelled do not have heart failure) and the patients are intensely heterogeneous. Imaging, particularly echocardiography, can provide extensive information regarding diastolic function, but diastolic dysfunction may certainly be present in the absence of heart failure. Myocardial tissue characterisation is potentially important in the distinction of aetiologies, including recognition of myocardial scar, which may be focal or diffuse. Stress imaging is insufficiently used in individuals where this diagnosis is suspected. First, coronary artery disease may present with dyspnoea alone, and second, the assessment of physiology during exercise seems prudent in order to make the diagnosis of a condition where symptoms are provoked by activity.

**Subclinical heart failure** - Patients at risk of heart failure (stage A) are classified as being in stage B heart failure if there is evidence of abnormal cardiac structure. The distinction is relatively simple in patients with ischaemic heart disease, but more challenging in non-ischaemic heart failure. The existing criteria for this exercise relate to recognition of left ventricular hypertrophy. CMR is substantially more reliable for this purpose than echo, although 3-D echo is a reasonable alternative. A number of abnormal functional markers have been considered as providing evidence of stage B heart failure, including myocardial strain and diastolic dysfunction.

Atherosclerosis
Although atherosclerosis is a universally recognised complication of diabetes, the application of imaging for this diagnosis is in some ways analogous to the discussion of heart failure.

**Overt atherosclerosis** - These conditions relate to classical descriptions of coronary, cerebrovascular and peripheral vascular disease as well as aortic aneurysm disease. Standard imaging approaches are used, with contrast angiography and CT remaining the mainstream tests, but functional testing to define ischaemia rather than just arterial disease remains important, particularly in the heart.

**Small vessel disease** - This comprises both structural and functional abnormalities of the small vessels, and is somewhat analogous to HFpEF in terms of the inadequacy of our diagnostic approaches. CMR offers some interesting insights, and the strongest evidence for this has been obtained for the diagnosis of chest pain with normal coronary arteries in women.

**Subclinical atherosclerosis** - Given the presentation of many patients with a catastrophic event such as sudden death, myocardial infarction or stroke, the recognition of early disease remains topic of much interest. The results to date with strategies for screening for CAD using functional testing have been disappointing. CT coronary calcium score has been shown to provide useful prognostic information in very large observational studies, but the benefit of intervention strategies based upon these findings remains to be proven.

93: NEW AND OLD DRUGS IN DIABETES AND HEART FAILURE

Burrell, Louise M
Department of Medicine, University of Melbourne, Austin Health, Melbourne, Australia

Diabetes and heart failure commonly coexist, and each adversely affects the prognosis of the other. Whilst improved glycemic control has been important in reducing morbidity and mortality, the impact of glucose-lowering drugs on heart failure outcomes is limited. Evidence for the use of commonly used drugs (i.e. metformin, sulfonylureas and insulin) comes from registries, observational data and subgroup analysis. The finding that thiazolidinediones were associated with adverse cardiovascular (CV) outcomes led to US FDA industry guidance for the licensing of new antidiabetic drugs. There are now > 170,000 patients with diabetes enrolled in ongoing CV outcome trials with dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide (GLP)-1 receptor agonists and sodium–glucose co-transporter 2 (SGLT2) inhibitors. There is renewed interest in augmenting the natriuretic peptide system in CV disease, and results from recent studies in heart failure showing CV benefits with agents that combine neutral endopeptidase inhibition with angiotensin receptor blockade will be discussed.
Although cardiovascular (CV) disease as a result of increased atherosclerotic burden remains the major cause of mortality in diabetes, most current treatments have not led to a major improvement in the prognosis of this condition. The major benefits appear to be related to non-diabetes specific effects of lipid lowering with statins. The ADVANCE trial and its follow up study, ADVANCE-ON have demonstrated limited benefits of improved blood pressure and glycaemic control in type 2 diabetes. Intensification of glycaemic control has been shown to reduce CV events in type 1 diabetes in the DCCT/EDIC trial but the effects take decades to become clinically evident.

Experimental studies using various models of diabetes associated atherosclerosis have identified not only the role of old targets such as the renin-angiotensin system but also newer targets including haemodynamic factors such as vasoconstrictors including endothelin and urotensin II but also glucose related pathways including the advanced glycation pathway (intermediates such as methylglyoxal, ligands such as AGEs and receptors such as RAGE) and various pathways implicated in reactive oxygen species (ROS) accumulation. This includes the enzyme, NADPH oxidase (Nox) where our group has shown atheroprotection with deletion of the Nox 1 isoform and use of novel Nox inhibitors. Furthermore, approaches to enhance antioxidant defense such as repletion of the enzyme Gpx1 with drugs such as ebselen appear to be promising.

In conclusion, there are a number of innovative approaches currently under investigation to reduce the burden of atherosclerosis in the context of type 1 and type 2 diabetes.

95: TREATMENT-FREE HYPERTENSION

Mills, Charlotte
Division of Diabetes and Nutritional Sciences at King’s College London, United Kingdom

Epidemiological evidence shows that the Mediterranean and Japanese traditional diets, and diets rich in green leafy vegetables per se, are associated with a low incidence of cardiovascular disease. The DASH study showed a direct effect of a fruit and vegetable diet on blood pressure. Attempts to identify individual components responsible for these effects have generally been inconclusive. An alternative approach recognises that these diets are rich in dietary (inorganic) nitrate. The discovery of dietary nitrate’s important vascular effects came from the relatively recent realization of the ‘nitrate-nitrite-nitric oxide (NO) pathway’. Dietary nitrate, mainly administered in the form of beetroot juice, has now been demonstrated to have a range of beneficial vascular effects in humans, including reducing blood pressure, inhibiting platelet aggregation, preserving or improving endothelial dysfunction, and enhancing exercise performance in healthy individuals, and patients with peripheral arterial disease. Pre-clinical studies reveal the potential of nitrate/nitrite to reduce arterial stiffness, vascular inflammation and intimal thickness. Pulse wave velocity (PWV), a measure of large artery stiffness, is an independent predictor of cardiovascular events. Our current study is investigating a potential mechanism for dietary nitrate, ingested as beetroot juice over 6 months, to reduce arterial stiffness in 120 patients with, or at risk of, diabetes.

96: IMPACT OF OBESITY AND INSULIN RESISTANCE ON SERUM DIHOMO Γ-LINOLENIC ACID

Matsuda M
Department of Cardiology, National Hospital Organization Kure Medical Center and Chugoku Cancer Center

Background: Diet therapy or supplementation that elevates eicosapentaenoic acid (EPA)/ arachidonic acid (AA) ratio or n-3/n-6 polyunsaturated fatty acid (PUFA) ratio is effective to suppress the incidence of diabetes as well as cardiovascular events. However, it is unclear how the n-6 PUFA synthesis is clinically regulated. Dihomo γ-linolenic acid (DHLA) is an intermediate metabolite of AA synthesis, which cannot be taken from natural food and need to be synthesized from the precursor n-6 PUFA, while the dietary intake, but not the de novo synthesis, is crucial for AA. The current study aimed to clarify the association between serum DHLA or AA levels and obesity or insulin resistance in non-diabetes patients without n-3 PUFA supplementation.

Methods: We conducted a cross-sectional observational study on 407 non-diabetes patients who were treated with statins. The patients treated with other anti-hyperlipidemic agents including fibrates, ezetimib and n-3 PUFAs, were excluded. We measured serum EPA, DHLA, and AA concentrations and investigated their relationship with body mass index (BMI), homeostasis model assessment-insulin resistance (HOMA-IR), and serum levels of various lipoproteins including remnant lipoprotein cholesterol (RemL-C).

Results: Single regression analysis revealed a strong correlation between serum DHLA and AA levels (r = 0.512, p < 0.0001). Serum DHLA levels were significantly associated with BMI (p < 0.001), while AA levels were not. In particular, the highest quartile group (HOMA-IR ≥ 2.0) showed significantly higher levels of DHLA than either lower quartile group. In multiple regression analyses, serum DHLA and AA levels were significantly correlated with log-converted RemL-C levels, independently of BMI and HOMA-IR, although the association of DHLA with RemL-C was statistically stronger than that of AA (β = 0.530, p < 0.0001; β = 0.273, p < 0.0001, respectively). In multiple regression analyses, DHLA levels, but not AA levels, were negatively correlated with EPA levels, independently of BMI, HOMA-IR and RemL-C levels (β = -0.275, p < 0.0001).
**AAS Session - YOUNG INVESTIGATOR SESSION**

**97: HIGH-DENSITY LIPOPROTEIN INHIBITS HUMAN M1 MACROPHAGE POLARISATION THROUGH THE REDISTRIBUTION OF CAVEOLIN-1**


Baker IDI Heart and Diabetes Institute, Melbourne, Australia; *University of Queensland, NZ*

**Background and Aims:** Macrophages play a critical role in the development and progression of atherosclerosis. Depending on their surrounding environmental milieu, macrophages can adopt a wide range of functional phenotypes; inflammatory (M1) and anti-inflammatory (M2). High-density lipoproteins (HDLs) have many cardio-protective properties including potent anti-inflammatory effects, largely through the removal of cholesterol from cells. It is currently not known if this extends to influencing human macrophage phenotypes. Thus, we aimed to investigate the effect of HDL on human macrophage polarisation.

**Methods:** Human blood monocyte-derived macrophages were induced to either an M1-phenotype by incubation with lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) or to an M2-phenotype with interleukin-4 (IL-4). Macrophages were differentiated in the presence or absence of human HDL and their phenotypes were characterised using cell surface markers and reactive oxygen species (ROS) production by flow cytometry, and mRNA expression by real-time PCR. Downstream signalling pathways were also explored.

**Results:** We discovered that HDL inhibited the induction to M1 as evidenced by a decrease in cell surface marker expression; CD192 and CD64. This was accompanied by a decreased expression of M1-associated inflammatory genes TNF-α, IL-6 and MCP-1. However, HDL had no effect on induction to the M2 phenotype. Similarly, methyl-beta-cyclohexane (MBCD), a non-specific cholesterol acceptor was able to suppress M1 induction suggesting cholesterol efflux is important in this process. Further, we found that HDL decreased membrane caveolin-1 in M1 macrophages and redistributed intracellularly. The requirement of caveolin-1 was revealed as bone marrow derived macrophages from Cav-1-/- mice continued to differentiate into M1 despite the addition of HDL. Moreover, we demonstrated a decrease in STAT3 and ERK1/2 phosphorylation in M1 macrophages treated with HDL, suggesting cholesterol efflux inhibits the STAT3s and MAPKs during induction to the M1 phenotype. Finally, we found that HDL also inhibited M1 function; with reduced ROS production.

**Conclusions:** We provide evidence that HDL reduces macrophage induction to the inflammatory M1 phenotype, but not M2, via cellular redistribution of caveolin-1 and inactivation of STAT3 signalling pathway.

**98: DEFICIENCY OF NOX4-DERIVED ROS PROMOTES A PRO-ATHEROGENIC PHENOTYPE IN MOUSE AORTIC SMOOTH MUSCLE CELLS EXPOSED TO HIGH GLUCOSE**

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**Introduction:** NADPH (Nox)- derived ROS play a central role in diabetes- accelerated atherosclerosis development and progression. Recently, Nox4 was identified to play an important role in maintaining the differentiated vascular smooth muscle cell (VSMC) phenotype. VSMC elaboration of signalling and extracellular matrix (ECM) proteins including platelet-derived growth factor (PDGF) and osteopontin (OPN) promote VSMC migration, proliferation and de-differentiation to a more synthetic phenotype. A potential link between Nox4-derived ROS and the downstream signalling pathways mediated by OPN and PDGF remains to be explored.

**Aims:** The aim of this study was to investigate the role of Nox4 in pro-atherogenic responses mediated by OPN and PDGF in mouse aortic smooth muscle (MASM) cells cultured under high glucose conditions. Methods: MASM cells were isolated from the aorta of ApoE KO and Nox4/ApoE DKO mice. Gene and protein expression was assessed by PCR, western blot and immunocytochemistry (ICC), respectively. Growth curve experiments were conducted to assess proliferation. Superoxide production was measured via L012 chemiluminescence in the presence or absence of the Nox1/ Nox4-dual inhibitor, GKT137831 (1µM).

**Results:** Nox4/ApoE DKO MASMs showed increased gene expression of OPN, PDGF and fibronectin with a concomitant decrease in aSMA gene and calponin protein expression levels compared to ApoE KO MASMs. Induction of OPN gene expression by PDGF was also enhanced in DKO cells cultured under high glucose conditions. Furthermore, DKO MASMs showed marked elevation in both intracellular and extracellular OPN protein expression in association with increased proliferation. Increased Nox1 mRNA levels in Nox4/ApoE DKO MASMs were coupled with increased superoxide production which was abrogated by GKT137831.

**Discussion/ Conclusion:** Nox4/ApoE DKO MASMs showed increased pro-proliferative and pro-fibrotic responses as well as decreased expression of contractility markers indicative of the atherogenic VSMC phenotype. Treatment of DKO cells with GKT137831 abrogated the observed increase in superoxide generation implicating Nox1 as the source of ROS in these cells. In this study we identify a role for Nox4 in the regulation of PDGF and OPN which may involve compensatory feedback via Nox1. These findings support a protective role for Nox4 in atherogenesis with respect to VSMC.
pathophysiology and highlight the importance of cell type and Nox isoform specific alterations in vascular disease.

99: APOLIPOPROTEIN A-I INCREASES GLUCOSE UPTAKE IN SKELETAL MUSCLE AND IMPROVES GLYCAEMIC CONTROL IN db/db MICE

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**Background:** Type 2 diabetes is associated with decreased plasma high density lipoprotein (HDL) and apolipoprotein (apo) A-I levels. Therapeutic interventions that elevate plasma HDL and apoA-I levels have been reported to reduce plasma glucose levels and attenuate insulin resistance. These observations suggest that HDL raising therapies may improve glycaemic control in type 2 diabetes.

**Objective:** This study asks if increasing plasma apoA-I levels improves glycaemic control in db/db mice by increasing glucose uptake in skeletal muscle, and investigates the underlying mechanisms of this effect.

**Methods:** Primary human skeletal muscle cells (HSKMCs) were incubated with or without apoA-I, in the presence or absence of insulin. HSMCs were also transfected with ABCA1 siRNA and SR-B1 siRNA prior to incubation with apoA-I. Activation of insulin signalling pathways was determined by western blotting and flow cytometry. Seven-week old insulin resistant male db/db mice were fasted for 3 h prior to administration of a single intraperitoneal injection of human apoA-I (40 mg/kg) or PBS. After 2 h the mice were randomly assigned into 3 groups and subjected to a glucose tolerance test, an insulin tolerance test and ex vivo glucose uptake by the gastrocnemius muscle.

**Results:** Incubation of HSKMCs with apoA-I increased insulin-dependent and insulin–independent glucose uptake in a time- and concentration-dependent manner. The increased glucose uptake was accompanied by enhanced phosphorylation of insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K), serine/threonine kinase Akt and Akt substrate of 160 kDa (AS160). Cell surface levels of the glucose transporter, GLUT4, were also increased. The apoA-I-mediated increase in glucose uptake by HSKMCs was dependent on the ATP binding cassette transporter A1 (ABCA1) and scavenger receptor class B type I (SR-BI). Treatment with apoA-I increased plasma apoA-I levels in db/db mice, significantly improved their glucose tolerance and insulin sensitivity and increased insulin-dependent and –independent glucose uptake by the gastrocnemius muscle.

**Conclusions:** ApoA-I increases glucose disposal in skeletal muscle by activating the IRS-1/PI3K/Akt/AS160 signal transduction pathway and improves glycaemic control in db/db mice. These findings suggest that therapeutic agents that increase plasma HDL and apoA-I levels may improve glycaemic control in people with type 2 diabetes.

100: APOA-I SUPPRESSES NEOINTIMAL HYPERPLASIA FOLLOWING STENT DEPLOYMENT AND MODULATES NEOINTIMAL CELLULAR PHENOTYPE

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**Background:** Increases in smooth muscle cell (SMC) cholesterol content reduce the expression of SMC phenotypic markers and increase macrophage related markers. The balance of neointimal SMCs to macrophage-like cells may influence neointimal hyperplasia following stent deployment. Apolipoprotein (apo) A-I, the main protein component of high-density lipoproteins (HDL), mediates the efflux of cholesterol from cells via the cholesterol transporter ABCA1. In a murine model of balloon angioplasty and stenting, we aimed to investigate the effect of apoA-I on neointimal hyperplasia and neointimal cellular phenotype.

**Methods and Results:** Thoracic aortic segments from apoE knockout mice underwent balloon angioplasty alone or balloon angioplasty with stenting then were carotid-interposition grafted into recipient mice. Mice received PBS or apoA-I (40mg/kg) injections on alternate days in the week prior to surgery and then until sacrifice (28 days). There were no changes in total cholesterol, LDL and HDL between groups at sacrifice and thrombosis rates were 16.7% lower in apoA-I injected mice. Histological analysis revealed that stented aortic sections from mice injected with apoA-I had significantly smaller neointimal areas (18%±6.9), compared to PBS control mice p<0.05. The percentage of SMC alpha-actin positive staining was strikingly higher in the neointimases of apoA-I stented arteries (76%±34.3, p<0.05), while RT-PCR analysis of balloon angioplasty injured aortas found mRNA levels of macrophage marker CD68 were lower in apoA-I infused mice (30%±8.9, p<0.01). Furthermore, mRNA levels of ABCA1 and the cholesterol efflux regulator PPAR-gamma were significantly higher in apoA-I balloon-injured aortic segments (30%±13 and 48%±20.7, respectively), p<0.05.

**Conclusion:** ApoA-I infusions reduce neointimal area following stent deployment. In apoA-I infused mice, the cells of the neointima had significantly higher SMC alpha-actin expression and lower macrophage CD68 expression. Mechanistically, this may be due to increased SMC cholesterol efflux as apoA-I increased the expression of ABCA1 and PPAR-gamma. These findings have significant implications for therapeutic modulation of neointimal hyperplasia following stent deployment.
101: A NOVEL SURVIVAL PATHWAY OF ERYTHROPOIETIN IN PROTECTION OF NEONATAL MYOCYTES FROM CELL DEATH DURING HYPOXIA/REPERFUSION INJURY

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Background: Acute myocardial infarction (AMI) is a major cause of premature mortality in developed countries and is largely associated with Ischemia/Reperfusion (I/R) injury, which irreversible damage is caused to myocytes during infarction. Erythropoietin (EPO) is a hematopoietic cytokine, and its receptor (EPOR) is shown to be present in tissues outside blood, including the heart. EPO also possesses a non-hematopoietic action, mediated through inhibition of apoptosis and appears to be essential for the tissue-protective effects.

Aims: EPO is strongly inferred to protect the cardiomyocytes from the reperfusion injury and our aim is to elucidate the cardioprotective effect and exact mechanism behind the cardioprotection.

Methods: Neonatal cardiomyocytes (NCM) were exposed to H/R (Hypoxia/Reperfusion) with or without pretreatment using 10, 15 and 20 U/ml of EPO. We determined apoptosis using the following assays. The cell viability was determined using MTT, apoptotic nuclei by Acridine orange and Ethidium bromide, reactive oxygen species (ROS) by Dichlorodihydrofluorescein Diametate (DCF-DA), Mitochondrial Membrane potential (ΔΨm) by Rhodamine-123 and activity of late apoptotic protease, Caspase-3 activity by Caspase-3 assay. The expression of p-Akt and p-p38 MAPK, BAD, XIAP and Cyt-c exit were analyzed by western blot.

Results: Viability was increased from 44.4% in H/R injured NCM to 83.5% of (20U/ml) EPO pretreated NCM. Control and EPO pretreated NCM were seen as bright green colour nuclei whereas H/R injured NCM showed some early and late apoptotic/necrotic nuclei. In control and EPO treated myocytes, Rhodamine-123 colocalized with DCF fluorescence only in the perinuclear region. While in H/R induced NCM, Rhodamin-123 colocalized with DCF fluorescence both in perinuclear and cytoplasmic region. DCF fluorescence in NCM subjected to H/R is more than the fluorescence in control and EPO pretreated cells. EPO increases the phosphorylation of p38 MAPK, Akt, BAD and XIAP compared to HR. EPO pretreated NCM showed decrease cytosolic expression and increased mitochondrial expression of cytochrome c but NCW without EPO pretreatment showed vice versa. EPO prevented the caspase-3 activations induced by H/R. Further myocytes blocked with Wortmannin and SB203580 showed increase caspase-3 activity and thus abolishes the protective effect of EPO.

Conclusion: Hence we conclude in this study for the first time that EPO maintains ΔΨm, exerts both anti-apoptotic and anti-necrotic effect in I/R injured NCM through modulation of Akt, p38 MAPK and downstream effectors of Akt.

102: EXPRESSION PROFILE OF ADHESION AND CHEMOKINE MARKERS ON MONOCYTE SUBSETS AND THEIR CORRELATION WITH ATHEROSCLEROTIC RISK

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Background and Aims: Adhesion and migration of monocytes are key steps in atherosclerosis as these cells differentiate into plaque macrophages, which impact on stability. In mice, monocytes are divided into two subsets; the Ly-6Chi which are actively recruited into inflamed tissue and the Ly-6Clo which home into non-inflamed tissues. Notably, the expression pattern of chemokine receptors on mouse monocyte subsets can be used to identify human monocyte subsets (classical, intermediate and non-classical). Although considerable overlap in expression profile exists, there are also differences that prevent findings in mice from being directly applied to humans. Here we examine monocyte subset adhesion and migration profile by examining surface marker expression and how this changes with atherosclerotic risk.

Methods: Blood samples were collected from donors with no known cardiovascular or other inflammatory conditions. Lipid profiles were measured and the expression of different adhesion and migration markers was assessed by whole blood flow cytometry. Comparisons were made between monocyte subsets and relative to donor lipid profile.

Results: In regards to adhesion molecules, selectins (CD62L and CD44) were highly expressed on classical monocytes whereas integrins (CD11c, CD18 and CD49d) were higher on intermediate monocytes except for CD11a, which was higher on non-classical monocytes. The differing expression of selectins and integrins on the monocyte subsets suggests that classical monocytes have higher rolling capacity while intermediate monocytes have higher firm-binding potential. In regards to chemokine receptors, CCR2 and CXCR2 (CD182) were higher on the classical than the other monocyte subsets. In addition, on the classical monocytes, we found that CCR2 expression correlated with cholesterol levels while CD182 inversely correlated with ApoA-1. These findings suggest that classical monocytes may have greater capacity to migrate with increased atherosclerotic risk.

Conclusion: While our findings indicate that (in humans) intermediate monocytes may adhere more to the endothelium than classical monocytes, as atherosclerotic risk increases, the classical subset may have a greater capacity to migrate. If, as in the mouse, CCR2 plays a key role in monocyte entry into plaques, then the classical monocyte population could also be key contributor to human atherosclerosis. Future studies are required to further delineate the functional capacity of the individual monocyte subsets to migrate into the plaque.
103: INTRINSIC ChOLESTEROL HANDLING CAPACITIES OF HUMAN MACROPHAGES DERIVED FROM DIFFERENT MONOCYTE SUBSETS

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Background: Macrophages play a significant role in the progression and/or resolution of atherosclerosis. Macrophages are derived from blood monocytes which in humans are comprised of three subsets; classical – CD14+CD16-, intermediate – CD14+CD16+ and non-classical – CD14dimCD16+. Macrophages are able to polarise into the inflammatory M1 and anti-inflammatory M2 phenotypes. The ability of macrophages from the different monocyte subsets to handle cholesterol and potentially affect the atherosclerotic lesion is not known.

Aim: To identify and characterise the cholesterol handling capabilities of macrophages from the three different subsets.

Methods: Primary human monocytes were differentiated into macrophages in the absence and presence of native low-density lipoprotein (nLDL) and polarized into M1 (LPS and IFN-γ) or M2 (IL-4). Flow cytometery, fluorescent microscopy and real time qPCR were used to determine phenotype and function.

Results: We observed the formation of lipid loaded macrophages when incubated with nLDL and confirmed that nLDL was taken up in its unmodified form. Macrophages were differentiated from the different monocyte subsets and characterised for their expression of cholesterol influx (CD36) and efflux (ABCA1) receptors. Macrophages derived from the non-classical subset had a lower expression of these lipid handling genes compared to the other monocyte derived macrophage subsets, suggesting a reduced capacity for cholesterol handling. Fluorescent nLDL imaging confirmed that the non-classical subset-derived macrophages accumulate less nLDL. M2 macrophages are suggested to be involved in the resolution of inflammation. M2 macrophages from the non-classical subset have increased cholesterol influx receptors but the efflux receptor remains low, suggesting they are primed to take up lipids. Analysis of LXR, a sterol-responsive transcription factor for cholesterol efflux genes including ABCA1, revealed that basal LXR expression in M2 macrophages is low. However, if stimulated with an LXR agonist all monocyte derived macrophage subsets are able to up-regulate efflux receptor expression.

Conclusion: Monocyte subsets give rise to macrophages with different abilities to handle cholesterol. Importantly, the M2 inflammation resolving macrophages, particularly those from the non-classical monocyte subset, are primed to take up lipids suggesting that these cells may play an important role in atherosclerotic lesion regression.

104: DISTRIBUTION OF M2 MACROPHAGES WITHIN MAJOR AND SUBREGIONAL AREAS OF THE ATHEROSCLEROTIC PLAQUE

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Background: Within the inflammatory milieu of the atherosclerotic plaque there are a variety of monocyte derived macrophage phenotypes, in particular the M1 and M2 forms. The M1 is pro-inflammatory and associated with plaque instability. Conversely, the M2 is considered anti-atherogenic due to its anti-inflammatory and wound healing properties. As such, M2 macrophages may be a key therapeutic target due to their potential to stabilize atherosclerotic plaques. However, many of the specific interactive relationships of M2 macrophages with their unique complex micro environments in the plaque are not known.

Aim: To determine the distribution of M2 macrophages within defined plaque geographical (sub)regions and identify possible pro- and anti-atherosclerotic functions that may impact on plaque stability.

Method: Formalin fixed paraffin embedded (FFPE) serial sections of human carotid plaques were stained by histochemical (HC) and immunohistochemical (IHC) techniques to define regions and identify cell subsets. Qualitative image analysis was performed on high resolution scanned slides.

Results: The M2 markers had an overlapping and ubiquitous distribution within the plaque, primarily admixed with an inflammatory infiltrate. Neither M2 marker was associated with plaque calcification. CD206 and Glycophorin A staining appeared to be matched and was associated with scattered hemosiderin deposition. This is consistent with previous studies identifying CD206 on Mhem macrophages in regions of intra-plaque haemorrhage. Single staining showed that within foam cell regions some cells stained for CD163 whilst others stained for CD206, which possibly reflects different functionality. The CD163 foam cells were found to be actively taking up lipid as seen by double staining with ADRP. As some foam cells were CD206 positive, this suggests that they had actively taken up lipid despite being in a region of haemorrhage. However, haemoglobin has been shown to limit lipid uptake in culture.

Conclusion: These preliminary results show that M2 macrophages have multifaceted / multifunctional roles within the plaque which are influenced by the dynamic micro environments. These pilot results need further investigation to help confirm the role of M2 macrophages within the (sub)regions and how these factors may contribute to plaque stability.
Accumulating evidence from mainly uncontrolled and unblinded clinical studies with various types of ablation catheters have shown that renal denervation (RDN) can be applied safely and is effective in lowering blood pressure (BP) in most patients with treatment resistant hypertension. Sustained BP lowering has been documented up to 3 years at this stage. Furthermore, RDN has been associated with regression of target organ damage, such as left ventricular hypertrophy, arterial stiffness and others. Several studies indicate potential benefit in other common clinical conditions associated with increased sympathetic tone including chronic kidney disease and heart failure. However, the recently published Symplicity HTN-3 trial, the largest and most rigorously designed clinical trial including a sham procedure, while confirming the safety of the procedure, failed to demonstrate a BP lowering effect beyond that of a sham procedure in a cohort of patients with resistant hypertension. Aspects of the trial that may account for the substantial discrepancy between Symplicity HTN-3 and other RDN studies will be discussed and the implications for future research in the area highlighted.

106: DOES DIET HAVE A ROLE IN SECONDARY PREVENTION?

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Except for the use of fish oil there have been very few randomised controlled trials addressing in diet in secondary prevention and all of these were done in the pre statin era or just as statins were appearing and all were small with few events. Certainly there is suggestive but not convincing evidence that changes in diet along with standard post-ischemic event treatment can reduce events. These diets encourage vegetables, fruit, fish, whole grains and olive oil along with reduced saturated fat, pattern very similar to the Mediterranean diet. In the REasons for Geographic and Racial Differences in Stroke study participants with CHD (n = 4,174) being in the highest versus the lowest quartile for Mediterranean diet adherence led to a 23% reduction in recurrent CHD and a 16% reduction in death but neither was statistically significant. These dietary effects were weaker than exercise 4 or more times /week and stopping smoking-32% and 50% reduction in CHD respectively and reductions in death were similar (booth 2014). Changes in waist circumference were not associated with outcomes, reflecting the absence of benefit of weight loss in primary prevention in the Look Ahead study. The use of fish oil alone as a dietary strategy has been abandoned by many authorities (eg in Europe) given the recent negative trials. Chocolate consumption is associated with lower cardiac mortality over an 8 year period after acute myocardial infarction in Stockholm. There may be confounding by anxiety and depression status, alcohol intake, drug compliance and other dietary components. More research is required.

107: TEN YEAR LEGACY EFFECTS OF BASELINE BLOOD PRESSURE TREATMENT NAIVETY IN THE SECOND AUSTRALIAN NATIONAL BLOOD PRESSURE STUDY

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Background: Guidelines now recommend that treatment thresholds for blood pressure (BP) be based on absolute cardiovascular disease (CVD) risk. Clinicians are concerned that leaving elevated BP untreated may lead to long-term adverse cardiovascular events.

Aims: To examine differences in CVD events at 10 years between those who entered a large-scale randomized controlled BP study (ANBP2) after withdrawing from existing long term therapy (‘on-treatment’) and those who were ‘treatment-naïve’ in order to help resolve this concern.

Methods: All participants entering the ANBP2 study who did not have a history of a CVD event at baseline were included in the analysis. Cox-regression hazard models, adjusted for clustering and for potential risk factors, were used to assess the effects of previous BP lowering medication use on different study endpoints within the ANBP2 clinical trial (such as any first cardiovascular events, stroke, myocardial infarction, heart failure, cardiovascular mortality and all-cause mortality). An extended 10 year follow-up analysis for cardiovascular and all-cause mortality was also conducted.

Results: We identified 5378 participants (aged 65–84 years; 52.5% women) who had no prior CVD events. No difference in fatal CVD events [hazard ratio (HR) 0.96 (95% CI 0.79–1.16); P=0.65] or all-cause mortality (0.96 [0.83-1.11]; P=0.58) between treatment-naïve and on-treatment groups was observed after a median of 10.8 years and therefore no long-term evidence of a legacy effect. We did find lower in-trial HR for fatal CVD events (0.50 [95% CI 0.27-0.81]; P=0.007) and all-cause mortality (0.63 [95% CI 0.46-0.86]; P=0.004) for the treatment-naïve compared to the on-treatment group. This was observed despite the treatment-naïve group having a poorer CVD risk profile. For looking for
temporal effects, we investigated whether this was an effect of the in-trial protocol, since those on-treatment had gone through a drug withdrawal program prior to randomization, but this did not explain the observed differences.

**Conclusions:** We found no evidence for long-term adverse CVD risk associated with delayed treatment of elevated BP in an elderly hypertensive cohort. Observed differences between the groups may suggest “healthy survivor” effects may be at play. Legacy effects need to be further explored in randomized placebo controlled trials of middle-aged populations as this is the population of clinical concern.


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**Background:** Serum NT-proBNP level predicts heart failure. The SCREEN-HF study is a community-based cohort study that aims to identify an appropriate threshold NT-proBNP level for stratification of individuals into high and low risk for heart failure.

**Aim:** To assess whether serum NT-proBNP level can predict heart failure risk in an at-risk population.

**Methods:** We recruited people with at least one risk factor for heart failure: age ≥60 years with one or more of self-reported myocardial infarction or other ischemic or valvular heart disease, arrhythmia, cerebrovascular disease, renal impairment, or treatment for hypertension or diabetes for ≥2 years. Exclusion criteria were known heart failure or left ventricular dysfunction on previous cardiac imaging. Blood was collected from all participants at baseline for measurement of electrolytes, creatinine and NT-proBNP. Median age of the 3938 participants (2171 men and 1767 women) was 70 years (interquartile range 65-75), 83% were receiving treatment for hypertension, 18% were diabetic, 23% had ischaemic heart disease (IHD), 11% had cerebrovascular disease, 10% had atrial fibrillation (AF), 32% had body mass index (BMI) >30 kg/m², 7% had obstructive sleep apnoea (OSA), and 23% had glomerular filtration rate <60 ml/min/1.73 m².

**Results:** At the time of this interim analysis there were 77 cases of incident heart failure (49 men and 28 women) during a median follow-up of 6 years (incidence rate 3.3 per 1000 person years). Relative to NT-proBNP tertile 1, the odds ratio for incident heart failure was 4.0 (95% confidence interval: 1.1–14.4) for tertile 2 and 21.6 (6.8–69.0) for tertile 3. The C-statistic from receiver operating characteristic analysis was 0.81 (0.77–0.86), with similar values for men and women. NT-proBNP >18 pmol/l (the highest 35%) predicted incident heart failure with 80.5% sensitivity, 66.2% specificity, positive predictive value 4.5% and negative predictive value 99.4%. Although age, diabetes, IHD, AF, BMI and OSA were significant predictors of incident heart failure in a multivariable logistic regression model including NT-proBNP, none improved classification of heart failure risk beyond NT-proBNP alone. Among 3046 participants who had echocardiography, NT-proBNP >18 pmol/l predicted left ventricular ejection fraction (LVEF) <45% with 74% sensitivity and LVEF <40% with 79% sensitivity.

**Conclusions:** Serum NT-proBNP level assists stratification of heart failure risk among a community population with risk factors for heart failure. Improved identification of individuals at increased risk of heart failure will enable targeting of preventative therapies.

109: ASPREE (ASPirin in Reducing Events in the Elderly): A PROGRESS REPORT

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**Rationale:** ASPREE is a large-scale (n=19,000) double-blind, placebo-controlled trial investigating whether low dose aspirin can extend disability-free survival in persons aged 70+ (65+ for US minorities) conducted in Australia and the USA, funded primarily by the NIH. The beneficial effect of low dose aspirin for the primary prevention of cardiovascular disease is controversial, despite the conduct of 6 randomized trials addressing the question. Equipoise remains as to whether reductions in myocardial infarction, ischemic stroke, cancer and cognitive decline outweigh the risks of hemorrhagic stroke and gastrointestinal bleeding events. The elderly, in particular, are the group in which evaluation of benefit and risk balance is critical.

**Aim:** To provide an update on progress with ASPREE.

**Methods:** Subjects free of known previous cardiovascular disease, no life threatening illness and with no contraindication to aspirin are randomized (1:1) to 100 mg enteric coated aspirin or matching placebo. The primary composite endpoint includes death from any cause, dementia or persistent physical disability. Secondary events include cardiovascular events and clinically significant bleeding. Community dwelling participants are being recruited through general practices in Australia and clinical trial centres in the USA where recruitment is focused on minority groups. The study commenced recruiting in March 2010 and will complete recruitment by December 2014.

**Results:** By Sep 2014, in Australia 2107 GPs have participated, 73,727 phone screenings yielded 19,420 eligible visits with 15,876 participants randomized. In the USA, 2289 participants have been recruited from 24 clinical sites with 54% minority recruitment. Across both countries, 56% of participants are women and % age distribution (years) is: 3% (65–69), 55% (70–74), 8% (75–79), 14% (80+). Following 33,583 years of follow-up, 0.7% of participants have withdrawn from the study, 82% remain either on medication or have temporarily ceased randomized therapy and 875 events (including cardiovascular events and clinically significant bleeding) have been adjudicated.

**Conclusions:** With >95% of recruitment achieved, high levels of retention and follow-up, and accumulating end-
points, ASPREE will be sufficiently powered to detect a 10% difference in the primary endpoint following an average of 5 years of follow-up. With an increased interest in the potential for aspirin to provide protection against the major diseases of aging, ASPREE is a unique study in the elderly and will contribute to resolving the equipoise regarding risks versus benefits in primary prevention.

**ISC2 Session - LOWERING CV RISK IN HIGH RISK POPULATIONS**

**110: ABORIGINAL POPULATION**

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Much is known about the poor health status of Indigenous Australians. Increasingly, the contribution of CVD to seemingly intractable health disparity for Indigenous Australians is being recognised. This presentation will focus on the burden of CVD and its determinants in disadvantaged Australians. In addition, a number of key targets for reform will be outlined, and recent efforts to close the gap in life expectancy for Indigenous populations through specific focus on cardiovascular and related conditions.

**111: FAMILIAL HYPERCHOLESTEROLEAMIA IN AUSTRALASIA AND BEYOND**

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The gaps and needs in the care of FH have been well recognised in Australasia. The FH Australasia Network developed a consensus model of care (MoC) for FH in 2011. The MoC is based on expert opinion, published evidence and consultations with many stakeholders, and was initially developed for use primarily by specialist centres. The MoC aims to provide a standardised, high-quality and cost-effective system of care that is likely to have the highest impact on patient outcomes. It encapsulates comprehensive recommendations on the standards required for the detection, diagnosis, assessment and management of FH in adults and children. The process involved in cascade screening and risk notification, the backbone for detecting new cases from a proband with FH, is detailed; the cost-effectiveness of genetic testing for FH was recently confirmed. Guidance on treatment is based on risk stratifying patients, management of non-cholesterol risk factors, and safe and effective use of statins. A comprehensive system for providing best clinical care is described, and an integrated model of care centre on family doctors (GPs) and primary care was recently developed. Our experience has informed an international MoC for FH, which in turn has laid the foundations for an investigation in the Asia-Pacific rim ("The 10-Countries Study"), funded by the International Atherosclerosis Society, that addresses several issues in FH (community prevalence, genetic testing, patient perceptions and experiences, physician awareness and practices, and service provision and facilities). A web based registry, comprised of 5 modules, that links the FH Australasia Network has also recently been developed.

References

**112: RENAL FAILURE / REDUCING RISK**

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Chronic kidney disease (CKD) is highly prevalent in the community, affecting 10-15% of adults in Western Countries. People with CKD have an increased risk of cardiovascular events and death, and a reduced quality of life. It is not clear whether the pathophysiology of cardiovascular events is altered in people with CKD, who may also have alterations in the excretion or metabolism of many agents, rendering them more susceptible to adverse effects.

Despite this clear need and the potential for differences in the risk-benefit ratio in people with CKD, they have been consistently excluded from many trials of potentially protective therapies, and the number of trials conducted specifically in this patient population has been relatively modest. In recent years, new evidence regarding the effects of proven CV protective strategies in people with CKD has emerged, helping to clarify the role of lipid lowering, BP lowering and antiplatelet therapy in this population. Additional data regarding the effects of CKD—specific approaches to CV risk reduction
has also emerged, including haemoglobin normalisation, PTH lowering and extended dialysis. These data will be reviewed in this presentation.

113: STATIN USE FOR “PRIMARY PREVENTION” DOUBLES WITH THE NEW “ACC/AHA GUIDELINE ON THE TREATMENT OF BLOOD CHOLESTEROL TO REDUCE Atherosclerotic Cardiovascular Risk”: COMPARISON OF TWO AMERICAN GUIDELINES

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Background and Aims: The new “2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic CV Risk in Adults” (ACC/AHA_gl) differs from previous guidelines in different aspects of hyperlipidemia management. In a sample representative of Turkish population, we compared (ACC/AHA_gl) with “Adult Treatment Panel (ATP) III” guideline (ATPIII_gl) in terms of statin recommendations for primary prevention

Methods: From a cross-sectional population, 2657 individuals (52.8% female) between 40 and 74 years old were included, as they had no known previous CV events and statin use. CV risk was calculated using “Global Framingham Risk Score Equations” (GFRSE) in ATPIII_gl and Pooled Cohort Risk Assessment Equations (PCRAE) (non-Hispanic Caucasians) in ACC/AHA_gl. “High risk” was defined as >=20% for “global” CV events and as >=7.5% for “hard” CV events in 10 years for PCRAE.

Results: Among traditional risk factors, LDL-c was 122±43 (female 126±47; male 118±43); HDL-c was 49±16 (female 52±16; male 46±15); triglyceride was 154±99 (female 152±98; male 156±100) and fasting blood glucose was 108±49 (female 111±54; male 106±45) mg/dL. Body mass index was 29.2 (female 30.4±5.4; male 28.0±4.2) kg/m2. The prevalence of smoking was 23.9% (female 9.5%; male 38.8%), diabetes mellitus was 21.6% (female 24.0%; male 19.1%), hypertension was 62.1% (female 67.5%; male 56.5%) and obesity was 41.8% (female 50.8%; male 32.5%). Proportion of the participants categorized as “high risk group” was 33.7% (23.0% of women and 44.8% of men) in GFRSE, and was 41.6% (26.7% of women and 57.1% of men) in PCRAE (p<0.001). According to ATPIII_gl 27.8% (27.0% of women and 28.6% of men) of the Turkish population were candidates for statin use. This proportion rose to 53.1% (43.3% of women and 62.8% of men) using ACC/AHA_gl (p<0.001).

Conclusion: Compared to ATPIII_gl recommendations, ACC/AHA_gl increased the candidates for statins in primary prevention extensively. This difference, which has substantial impact on health economics, was more prominent in males. A national risk prediction model for Turkish population is needed and current guidelines should be interpreted cautiously in the context of efficacy, safety and cost effectiveness.

114: FXYD1 PLAYS A PROTECTIVE ROLE AGAINST MYOCARDIAL FIBROSIS

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Introduction: Fibrosis plays an important role in cardiac pathophysiology. We have demonstrated FXYD1 to be an endogenous protector of the cardiac membrane Na+•K+ ATPase against glutathionylation of its b1 subunit and oxidative inhibition. However, the effects of FXYD1 on longer term cardiac signalling, and particularly cardiac fibrosis has not been explored.

Aims: We aimed to determine the effect of FXYD1 on Angiotensin II (Ang II)-mediated cardiac fibrosis.

Methods and Results: In vivo studies were performed using wild-type (WT) and FXYD1 knockout (KO) mice. Ang II (1mg/kg/day) was infused via subcutaneous osmotic mini-pump for 1 week. Heart tissues were collected. As expected, Ang II induced interstitial fibrosis (151% of WT baseline +/-20%) and perivascular fibrosis (162% of WT baseline +/- 19%) in WT mice. Interestingly, FXYD1 KO resulted in an increase in both interstitial and perivascular fibrosis under baseline conditions (190% of WT baseline +/-15% and 215% of WT baseline +/-12% respectively), and profoundly augmented the Ang II effect (449% of WT baseline +/-14% and 472% of WT baseline +/-14% respectively). Parallel changes in fibrosis markers, TGF-b1, fibronectin and connective tissue growth factor expression were also observed, with the greatest levels of all three markers occurring in Ang II-infused FXYD1 KO mice. Given Src kinase activation has been shown to be coupled to changes in Na+•K+ ATPase conformation, and b1 subunit glutathionylation was increased by Ang II and FXYD1 KO, we examined Src kinase phosphorylation. Src phosphorylation (reflecting activity), again paralleled the differences seen above, being greatest in the Ang II-infused FXYD1 KO mice.

Discussion: These findings define a new role for FXYD1 as a protector of cardiac fibrosis from Ang II. Although the causal relationship needs further study, it is likely that FXYD1 protection against Na+•K+ pump b1 subunit glutathionylation, and subsequent Src activation is playing an important role in this novel pathway.
115: NOVEL ACTIONS OF SERELAXIN IN THE VASCULATURE

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The peptide hormone relaxin is a renal and systemic vasodilator, increases global arterial compliance and cardiac output, and mediates these parameters during pregnancy in conscious rats. These animal studies and later preclinical studies in compensated heart failure patients, which showed that relaxin decreases pulmonary capillary wedge pressure and systemic vascular resistance, suggested that relaxin treatment causes rapid vasodilation to improve the symptoms of heart failure. A recent phase III clinical trial (RELAX-AHF) demonstrated that a 48-hour intravenous infusion of serelaxin (recombinant human relaxin-2) to patients admitted to hospital with acute heart failure (AHF) resulted in significant beneficial clinical outcomes. Specifically, serelaxin improved dyspnoea relief, reduced in-hospital worsening of AHF and decreased cardiovascular and all-cause mortality at 180 days. I will present data on the mechanisms of serelaxin action in blood vessels and show prolonged vasorelaxant responses after an acute intravenous injection of serelaxin. These prolonged vascular responses after short-term serelaxin treatment have important clinical implications in the preservation of systemic vascular and organ function in AHF patients.

116: OBESITY-RELATED HYPERTENSION: INTERACTION OF THE IMMUNE AND SYMPATHETIC NERVOUS SYSTEMS

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Obesity-related hypertension is associated with increased sympathetic outflow. This increase in sympathetic activity is suggested to occur as a consequence of sympathetic hyperinnervation over the surface of blood vessels important for controlling blood pressure. Inflammation related to obesity may also influence sympathetic mechanisms regulating blood pressure. Using a range of techniques including pressure myography, electrophysiology, immunohistochemistry, FACS and plethysmography we have investigated role of sympathetic hyperinnervation and vascular inflammation in the development of obesity-related hypertension. We have shown that HFD initiates the rapid infiltration of NGF-positive immune cells to the vascular adventitia promoting sympathetic nerve growth. Furthermore, the adoptive transfer of T cells to Rag1\textsuperscript{-/-} mice fed a HFD restores obesity-related hypertension; while the transfer of T cells deficient in NGF (TcellT29A) does not. The immune cell driven increases in sympathetic nerve density augments nerve-mediated contractions and elevates blood pressure in obesity.

117: MATERNAL OBESITY AND THE DEVELOPMENTAL PROGRAMMING OF HYPERTENSION: THE ROLE OF LEPTIN IN THE CENTRAL NERVOUS SYSTEM

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Background: Obesity is a serious global disease burden. The prevalence of obesity in women of child bearing age is increasing and this has been reported to be parallel to the increase in obesity in the general population. Aim: To determine the ability of trans-generational “programming” of leptin signaling in the central nervous system (CNS) to increase blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) following an initiation of a high fat diet (HFD) in mothers. Methods: Female breeder New Zealand White rabbits were fed a high fat (13%) diet (mHFD) or a normal fat diet (mNFD), 3 weeks prior to mating, during pregnancy and lactation. Kittens from mNFD rabbits were subdivided and fed with HFD for 10 days (mNFD10dHFD) at 15 weeks of age. All rabbits received an intracerebroventricular (i.c.v.) catheter into the lateral ventricle and a recording electrode was placed on the left renal nerve. Experiments were conducted in conscious rabbits at 16–18 weeks of age and BP, HR and RSNA was measured. Rabbits received increasing doses of melanocortin 3,4 receptor antagonist (SHU9119), α-melanocortin stimulating hormone (αMSH) and a single dose of leptin antagonist by i.c.v. infusion on separate days. Results: At baseline measurement, mNFD10dHFD rabbits had higher BP and RSNA compared to mNFD or mHFD rabbits (P<0.001). ICV injection of SHU9119 reduced BP, HR and RSNA in all rabbits that were exposed to HFD (mHFD and mNFD10dHFD; P<0.001). Leptin antagonist reduced BP, HR and RSNA but only in mHFD rabbits. αMSH led to increased BP, HR and RSNA in both mHFD and mNFD10dHFD rabbits (P<0.05). Total % fat was markedly higher in all rabbits that were exposed to HFD. Conclusions: Obesity during pregnancy programs the leptin signaling pathway in the CNS of the offspring during development. Leptin, via activation of melanocortin pathways, plays a key role in the CNS by contributing to the pressor and tachycardic effects as well as renal sympathetic nerve activity in the pathophysiology of obesity.
**Background:** Inflammation and fibrosis in blood vessels and the kidneys play key roles in the pathophysiology of hypertension. IL-1β is a cytokine crucial for the initiation of inflammation and subsequent tissue damage in several chronic diseases. Its role in hypertension has not, however, been investigated.

**Aims:** To investigate whether treatment with an IL-1 receptor antagonist (anakinra) reduces renal and vascular inflammation in deoxycorticosterone acetate (DOCA)/salt-induced hypertension in mice and whether this is associated with a reduction in systolic BP.

**Methods:** Hypertension was induced in mice by uninephrectomy, treatment with DOCA (2.4 mg/d, s.c.) and replacement of drinking water with saline (0.9%). Control mice received uninephrectomy and a placebo pellet (s.c.). At day 10 post-surgery, mice underwent further treatment with either anakinra (75 mg/kg/d, i.p.) or vehicle (0.9% saline, i.p.) for 11 days. Systolic BP was measured via tail cuff and, at day 21 post-surgery, mice were killed and their kidneys and thoracic aorta removed for ex-vivo analyses. Real-time PCR was used to determine mRNA levels of pro-inflammatory cytokines (IL-1β, IL-18, IL-6) and other markers of inflammation (ICAM-1, VCAM-1, CCL5, CCL2). Renal fibrosis was determined by picrosirius red staining.

**Results:** At day 10 post-surgery, systolic BP was elevated in DOCA/salt-treated mice (150.2±2.8 mmHg) compared to control mice (121.3±3.3 mmHg; n=16, P<0.001). Treatment with anakinra partially reversed the pressor effect of DOCA/salt treatment such that by day 14, BP had dropped by ~20 mmHg (n=8, P<0.01). By contrast, vehicle treatment had no effect on BP in DOCA/salt-treated mice. Real-time PCR showed that IL-1β level was elevated in kidneys of DOCA/salt-treated compared to control mice, as were mRNA levels of other inflammatory genes, including IL-6, ICAM-1, VCAM-1, CCL5 and CCL2 in both the kidneys and aorta (n=7–9, P<0.05). Importantly, treatment with anakinra partially reduced the level of several of these including CCL5 and CCL2 in the kidneys (n=7–9, P<0.05) and ICAM-1, VCAM-1, and CCL2 in the aorta (n=7–8, P<0.05). Finally, collagen deposition was increased in the kidneys of DOCA/salt-treated mice compared to controls (n=8, P<0.01), and again this was reversed by ~65% with anakinra treatment (n=6, P<0.01).

**Discussion:** Inhibition of IL-1β signaling in DOCA/salt-treated mice was effective at reducing renal and vascular inflammation, fibrosis, and lowering blood pressure, so highlighting this pathway as a potential target for future therapies.

**AAS Session - LIPIDS**

**119: 20 YEARS ON FROM 4S: HAVE WE SORTED OUT LDL?**

Nicholls, Stephen

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In 1994 the first large outcome trial reported that administration of simvastatin to survivors of myocardial infarction with elevated LDL-C levels had a profound impact on cardiovascular morbidity and mortality. Subsequent randomised controlled trials and meta-analyses have unequivocally demonstrated that lowering LDL-C is beneficial across a broad range of vascular risk and that this benefit continues to be observed with intensive LDL-C lowering. However, despite increasing need for use of statins with updated guidelines, many patients can’t tolerate these agents and a substantial residual risk of clinical events is observed. Increasing attention has focused on the role of particle measures of LDL and their implication for risk prediction and prevention. Novel approaches to LDL-C lowering in addition to statin therapy provides ongoing hope that there may be further reductions in risk, although this remains to be determined by clinical trials.

**120: HOW HIV (OR PARTS OF THEREOF) CAUSES DYSLIPIDAEMIA AND ATHEROSCLEROSIS**

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Baker IHi Heart and Diabetes Institute, Melbourne, Australia

HIV resides in limited number of very specific cell types, but complications of HIV disease include metabolic abnormalities in tissues not infected by the virus. We hypothesized that viral proteins secreted from infected cells impair metabolism in uninfected cells and systemically. We investigated the effect of Nef, a secreted HIV protein responsible for the impairment of cholesterol efflux, on the development of atherosclerosis in two animal models. In apoE−/− mice model Nef increased the size of atherosclerotic lesions and caused vessel remodelling. Nef caused elevation of total cholesterol and triglyceride levels in the plasma, while reducing high-density lipoprotein cholesterol levels. In C57BL/6 mice on high fat/high cholesterol diet Nef caused a significant number of lipid-laden macrophages presented in adventitia of the vessels. Nef caused elevations of plasma triglyceride levels and obesity. Our findings suggest that Nef causes dyslipidemia and accumulation of cholesterol in macrophages within the vessel wall, supporting the role of Nef in pathogenesis of atherosclerosis in HIV-infected patients.
121: HDL-SMALL RNA INTERCELLULAR COMMUNICATION AND ATHEROSCLEROSIS

Vickers, Kasey
Vanderbilt Univ. School of Medicine

High-density lipoproteins (HDL) serve as a general cargo carrier for a wide-variety of proteins, nucleic acids, and small molecules which likely confer many of HDL's alternative functions and anti-atherogenic properties. Circulating microRNAs (miRNA) on HDL originate from many types of cells, including macrophages. Using bone marrow transplant studies, we mapped the biodistribution of HDL-miRNA communication and identified a diverse set of recipient cells/tissues where inflammatory cell-originating HDL-miRNAs regulate target gene expression. Here we define key HDL-miRNA networks that contribute to cholesterol homeostasis, inflammation, and atherosclerosis. Moreover, we demonstrate that HDL-miRNA communication can be altered to inhibit atherogenesis and induce atherosclerosis regression. Strikingly, high-throughput small RNA (smRNA) sequencing has revealed that miRNAs are only a small fraction of RNA on HDL, as we found that HDL transport and deliver many other types of functional smRNAs that may also contribute to cardiovascular disease.

122: CELLULAR CHOLESTEROL HOMEOSTASIS IS ALTERED IN MURINE MODELS OF RHEUMATOID ARTHRITIS

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Baker IDI Heart and Diabetes Institute, Melbourne, Australia; *Walter and Eliza Hall Institute of Medical Research; Royal Melbourne Hospital

Background and Aims: Rheumatoid arthritis (RA) is associated with a ~2-fold elevated risk of morbidity and mortality from atherosclerotic cardiovascular disease (CVD) compared with the general population. Atherosclerosis in RA patients tends to be more aggressive and therefore more challenging to treat. Identifying CVD in these patients is difficult as traditional CVD risk factors, such as changes in plasma lipid profiles (i.e. elevated LDL, decreased HDL) are not always observed, underscoring the need for better understanding of the reasons contributing to the enhanced atherosclerosis in RA patients. People with RA often have monocytosis and neutrophilia, which can play causal roles in atherosclerosis.

Methods: Two mouse models of RA were used, K/BxN serum transfer and collagen induced arthritis (CIA). Flow cytometry was used to quantify the abundance of leukocyte and stem cell subsets. BODIPY-cholesterol was employed to determine the membrane cholesterol status of the various cells.

Results: Prominent monocytosis and neutrophilia due to an expansion and increased proliferation of the haematopoietic stem and multipotent progenitor cells (HSPCs) in the bone marrow (BM) was observed in both models of RA. There was also an increase in the mobilisation of HSPCs into the circulation, which homed to the spleen, resulting in extramedullary haematopoiesis. Interestingly, key cholesterol efflux genes, Abca1, Abcg1 and Apoe were down regulated in the BM HSPCs isolated from the K/BxN mice, resulting in increased cell membrane cholesterol levels. We also observed an increase in the expression of the common beta subunit of the interlukin-3 receptor on the HSPCs, likely explaining their increased proliferation. Moreover, blood monocytes and neutrophils had increased membrane cholesterol content, independent of changes in plasma cholesterol levels.

Conclusion: These data suggest that while plasma cholesterol levels may not be increased in RA, cellular cholesterol homeostasis might be increased. We hypothesize that this, together with enhanced monocyte production, underlies the increased risk of CVD in RA.

123: LIPOPROTEIN(A) STIMULATES ABCA1 IN THE LIVER CELLS VIA SCAVENGER RECEPTOR-B1

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Background and Aims: Elevated levels of lipoprotein(a) [Lp(a)] are an independent risk factor for developing cardiovascular disease (CVD). The association between Lp(a) and CVD risk shows a J-shaped curve suggestive of a protective effect at low Lp(a) levels. Some trials have reported a positive association between Lp(a) and high density lipoproteins (HDL). Whether Lp(a) has a direct effect on HDL is not known. Here we aimed to investigate if Lp(a) had any effect on the ATP binding cassette 1 (ABCA1) pathway of HDL production in liver cells.

Methods: Lp(a) was purified from the pooled plasma of five healthy individuals with high Lp(a) levels (>1000 mg/L). Low density lipoprotein (LDL) was also isolated and used as a positive control since it is known to upregulate ABCA1. HepG2 cells were treated with increasing concentrations (1 μg/ml to 10 μg/ml) of either purified Lp(a) or LDL. mRNA and protein levels of liver X receptor (LXRα), peroxisomal proliferator activated receptor (PPARγ) and ABCA1 were quantified by qPCR and western blot analysis respectively. Cholesterol efflux assays were also performed on Lp(a) treated cells. Lp(a) uptake by HepG2 cells was analysed by confocal microscopy, western blots and differential labelling of the lipid and protein components of Lp(a). The effect of blocking the scavenger receptor-B1 (SR-B1) on Lp(a) uptake, PPARγ, LXRα and ABCA1 mRNA and protein levels was investigated.

Results: Treatment of HepG2 cells with up to 5 μg/ml Lp(a) increased PPARγ, LXRα and ABCA1 expression. LDL also stimulated ABCA1 expression but to a lesser extent and not in a concentration dependent manner. The
upregulation of the ABCA1 pathway by Lp(a) was accompanied by an increase in cholesterol efflux to apoA1. A combination of confocal microscopy, western blots and differential labelling of the lipid and protein components of Lp(a), showed Lp(a) to be internalised by HepG2 cells. Internalisation of the lipid component of Lp(a) was blocked with an anti-SR-B1 antibody and this was associated with a marked attenuation in the ability of Lp(a) to upregulate, PPARγ, LXRα and ABCA1.

Conclusions: Lp(a) upregulates the PPARγ-LXRα-ABCA1 axis and promotes cholesterol efflux from liver cells. The ABCA1 response to Lp(a) is mediated by the selective uptake of Lp(a) lipids via SR-B1 rather than holoparticle uptake. We conclude that there is a biological connection between Lp(a) and HDL through the ability of Lp(a) lipids to upregulate the HDL biosynthesis pathway.

**ISCP Session - TARGETING THE OBESITY EPIDEMIC**

**124: PHARMACOTHERAPY FOR OBESITY: IS THERE ANY HOPE?**

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The track record of obesity pharmacotherapy has to date been chequered. The centrally acting drugs affecting noradrenergic and serotonergic systems, Dexfenfluramine and Sibutramine, were withdrawn because of adverse cardiovascular effects. The cannabinoid type 1 receptor antagonist Rimonabant was withdrawn due to depression and suicidality. Phentermine which has an amphetamine-like effect remains on the market with a label that limits prescribing to periods of three months and cautions about use in those at high cardiovascular risk. The peripherally acting intestinal lipase inhibitor orlistat has limited efficacy and inconvenient side-effects. Topiramate an antiepileptic with limited carbonic anhydrase activity showed good efficacy in initial trials but these were discontinued due to an unfavourable side-effect profile. Recently a number of new drugs have been approved for use in the USA by the FDA, these include: An extended release preparation of topiramate and phentermine each in a relatively low dose produces approximately 10% weight loss sustained at two years with only mild side-effects; Lorcaserin a selective serotonin receptor agonist has a more favourable side-effect profile but produces more modest weight loss; a sustained-release combination of the opioid antagonist naltrexone and bupropion (used as an antidepressant and for smoking cessation); and the long acting GLP1 agonist, liraglutide. The efficacy and side effects of these drugs are variable both within and between patients. Obesity is not a homogeneous condition and real-world use does not necessarily reflect what occurs in clinical trials. The optimal way to personalise the use of these drugs and the long-term risk benefit ratio are unknown. The results of longer term cardiovascular outcome trials are awaited.

**125 NEW APPROACHES FOR OBESITY: EFFECTS ON CARDIOVASCULAR OUTCOMES**

Nicholls, Stephen

Vascular Research Centre, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, Australia

In recent years a number of pharmacological and surgical approaches have been developed to specifically target obesity. These interventions have been reported to have favourable effects on adiposity related measures and associated metabolic risk factors. However, the ultimate impact of these approaches on atherosclerotic disease and cardiovascular outcomes will ultimately determine their clinical utility. The early findings of cardiovascular outcome trials of obesity targeted interventions and their regulatory implications will be reviewed.

**126: OBSTRUCTIVE SLEEP APNEA AND CARDIOVASCULAR DISEASE**

Antic, Nick

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Obstructive Sleep Apnea (OSA) is a highly prevalent disease. It is tightly linked to obesity so OSA is becoming more prevalent in our society. There is continually emerging evidence that OSA is a risk factor for CVS disease. OSA is linked to hypertension, atrial fibrillation, heart failure cerebrovascular disease and ischemic heart disease. The data around these associations will be presented and discussed. More recently evidence has accumulated that by treating OSA CV outcomes can improve. CPAP therapy and weight loss produce the most consistent results and when the two therapies are combined patient outcomes are better. A number of recent large RCTs assessing the efficacy of CAP or weight loss in treating OSA and reducing CV events will be discussed including some Australian studies that are still ongoing that have been designed to specifically answer the question can long term CPAP reduce the incidence of cardiovascular events.
AVBS/AAS/HBPCRA Session - THE IMMUNE RESPONSE IN CARDIOVASCULAR DISEASE

127: ENHANCE MYELOID CELL GLUCOSE UTILIZATION IN ATHEROSCLEROSIS

Yvan-Charvet, Laurent
INSERM, Paris, France

Atherosclerosis is a chronic inflammatory disease that is driven by the accumulation of macrophage foam cells in the artery wall. Although extensive research has focused on elucidating the roles of cytokines and the microenvironment in the migration, proliferation, differentiation and apoptosis of monocytes and macrophages, the metabolic pathways that regulate this process are not well understood. Detection of inflamed atherosclerotic plaques can be visualized by non-invasive 18FDG PET-CT imaging and this correlates with macrophage accumulation and inflammation. We recently reported enhanced glucose consumption in proliferating immature myeloid cells and in inflammatory macrophages in a glucose transporter 1 (Glut1)-dependent fashion. In an attempt to test the causal relationship between modulation of myeloid glucose utilization, inflammatory activation of myeloid cells and the development of atherosclerotic lesions, we carried out BM transplantation from mice with Glut1 deficiency or carbohydrate-responsive element-binding protein (ChREBP) deficiency, a transcriptional regulator of glucose.

128: GM-CSF ACTIVATES CARDIAC INFLAMMATION DURING KAWASAKI DISEASE

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1Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia, 2The Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia.

Kawasaki Disease (KD) is a leading cause of acquired pediatric heart disease. Disease pathology is characterized, and most likely initiated, by the infiltration of neutrophils and monocytes into the coronary arteries. The factors that drive cell migration into the heart remain obscure. To examine this issue, we examined which cytokines are involved in initiating cardiac inflammation during a murine model of KD (induced by a water soluble cell wall fraction of Candida Albicans, CAWS). We evaluated inflammatory cell infiltrate into the heart throughout the course of CAWS-induced vasculitis by flow cytometry. Using genetic and antibody neutralisation approaches, we identified an essential role for GM-CSF in triggering cardiac vasculitis. Surprisingly, we found that GM-CSF was produced by non-haemopoietic cells in the heart soon after administration of CAWS, and acts upon local hematopoietic cells to activate neutrophil recruitment into the heart. Our findings describe a novel role for GM-CSF in initiating cardiac inflammation, and implicate GM-CSF as a potential target for therapeutic intervention in KD.

129: MECHANISMS OF SYSTEMIC IMMUNE SUPPRESSION

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Infectious complications are the leading cause of death in the post-acute phase of stroke. Increased bacterial and viral infections in stroke patients suggest that the immune response, which normally eliminates these infections is inhibited in some fashion after such brain injury.

Indeed, there is accumulating evidence to indicate that brain injury, through the release of neurotransmitters, modulates the function of the immune system resulting in immune suppression, breakdown of immunological barriers and, consequently, infection. In fact, stroke injury triggers profound systemic immune effects, including severe reductions in the number of circulating lymphocytes and altered lymphocyte and monocyte function. It has been proposed that this immune suppression is a compensatory response to protect the post-stroke brain from overwhelming inflammation, but a side-effect of this is increased susceptibility to infection. Despite this, the molecular mechanisms whereby stroke mediates systemic immunosuppression have been elusive until very recently. Emerging evidence indicates a more selective modulation of the immune system following stroke could be beneficial. In fact, selecting facets of the immune system to target would allow the protective and regenerative properties of the immune response to remain intact while blunting the pro-inflammatory response generated towards the injured brain.
130: RAAS INHIBITION AND HEART FAILURE: THE END OR THE BEGINNING OF A NEW ERA?

Martinez, Felipe
Córdoba, Argentina

RAAS inhibition has been developed in the past 50 years and most of the investigation have shown beneficial results in cardiovascular disorders, mainly hypertension and heart failure. ACEis and ARBs have demonstrated consistent evidence in improving outcomes including a significant reduction of mortality. More recently, aldosterone blockade appeared to have a similar benefit in a wide spectrum of patients with heart failure and also additional improvement in resistant hypertension. Very recently two different approaches are arising in the horizons:
1)The increasing role of the Mineralocorticoid Receptor (MCR) in the physiology and pathogenesis of CV diseases, and the implications of its intervention, has already become a new strategy: MCR Blockade. Several consistent studies have demonstrated the important benefit of this new therapeutic tool, clearly linked to aldosterone antagonism and therefore to new RAAS inhibition
2)The revival of the “Dual RAAS inhibition” with LCZ696 (ARNI: Angiotensine Receptor Neprelisin Inhibitor) has proved in the PARADIGM Trial, a significant superior effect in mortality compared with enalapril, used in population with heart failure. The scientific figures are so significant that may allow to think we are facing a new era in this field.

131: HEFREF (HF WITH REDUCED EF)

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Over the last 2-3 decades, survival gains related to the management of heart failure have largely been in patients with a reduced left ventricular ejection fraction (HFREF). Pharmacological approaches that antagonise upregulated neurohormonal systems, specifically angiotensin converting enzyme (ACE) inhibitors (or angiotensin receptor blockers if ACE inhibitor intolerant), beta blockers and mineralocorticoid receptor antagonists reduce mortality and heart failure hospitalisations, and are indicated in potentially all patients with HFREF. Ancillary pharmacological options that improve long-term clinical outcomes in selected patients include sinus node inhibition with ivabradine, n3-polyunsaturated fatty acids and hydralazine/ nitrates. Device therapies (implantable cardioverter defibrillator, biventricular pacing) should be considered in patients with persistent adverse remodelling (LVEF <30-35%) despite pharmacological therapy. Recently, an angiotensin receptor nepriylisin inhibitor was shown to be superior to ACE inhibitor therapy in HFREF, which challenges the traditional treatment paradigm and demonstrates added benefit of augmenting natriuretic peptides in concert with angiotensin receptor blockade.

132: NOVEL INTERVENTIONS FOR HEART FAILURE

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Novel Interventions for Heart Failure: Many inroads into the pharmacological management of heart failure have seen therapies directed at modifying the neuro-humoral system lead to significant reductions in mortality and improvement in symptomatology. This includes treatments such as ACE inhibitors, beta blockers and aldosterone antagonists to name some of the more prominent. However, there remains a large unmet clinical need still in patient’s with heart failure. This presentation with review some of the novel treatment strategies, emerging in this space, from the current status in cell based therapies through to what’s new with natriuretic peptides.

HBPRCA Session - FREE COMMUNICATIONS

133: EFFECTS OF BLOOD PRESSURE LOWERING ON CARDIOVASCULAR RISK ACCORDING TO BASELINE BODY MASS INDEX: A META-ANALYSIS OF RANDOMIZED TRIALS

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Background: Recent reports have suggested that the benefits of blood pressure lowering in obese people compared to people of normal weight may depend upon the choice of drug.
Aim: To confirm or refute these findings from individual studies by conducting a meta-analysis based upon individual patient data using multiple trials included in the Blood Pressure Lowering Treatment Trialists’ Collaboration.
Methods: We compared the effects of blood pressure lowering regimens based upon different drug classes for the primary outcome of total major cardiovascular events. Meta-analyses and meta-regressions were used to seek evidence for interactions between treatment and body mass index (BMI) when fitted as either a categorical (<25, 25-30, > 30 kg/m²) or a continuous variable.

Results: Analyses were performed on 135,715 individuals drawn from 22 trials who experienced a total of 14,353 events. For none of the six primary comparisons made was there evidence that protection varied by drug class across the three BMI groups studied (all P for trend >0.20). When analysed as a continuous variable there was a slightly greater protection for each 5 kg/m² elevation in BMI when using an ACE inhibitor compared to a calcium antagonist (7%; 95% CI 2–11%; P=0.004) or a diuretic (7%; 95% CI 2–11%; P=0.002). The meta-regressions identified no relationship between BMI category and the risk reduction achieved for a given fall in systolic blood pressure. In contrast to a major prior report, we found no relationship between BMI and the efficacy of calcium antagonists compared to diuretics.

Conclusion: These analyses provide little evidence that the selection of a particular class of blood pressure lowering drug will substantially alter outcomes for individuals who are obese compared to those who are lean. (Publication: Lancet: in press)

134: ANGIOTENSIN CONVERTING ENZYME 2 DEFICIENCY PROMOTES AORTIC ANEURYSM FORMATION AND RUPTURE IN APOLPOROTTEIN E-DEFICIENT MICE


Background: Angiotensin (Ang) II has been implicated in aneurysm development and rupture. Angiotensin converting enzyme 2 (Ace2) is the major enzyme that metabolizes Ang II within vascular tissue. The expression of Ace2 is down-regulated at least 7-fold in the aortae of patients with abdominal aortic aneurysms when compared to healthy tissue from donors (P=0.025). We hypothesize that this down-regulation may contribute to the development and progression of aortic aneurysms.

Aim: To determine whether down-regulation of Ace2 mRNA in aorta contributes to development and progression of aortic aneurysms.

Methods: To explore this hypothesis, apolipoprotein (Apo) E gene knockout (KO) mice and ApoE/Ace2 KO mice were treated with Ang II (1 µg/kg/min, s.c.) or vehicle.

Results: At baseline, ApoE/Ace2 KO mice had larger aortic arch and suprarenal aortic diameters. The mRNA levels encoding proteins associated with inflammation and aneurysm formation were increased in the aortae of ApoE/Ace2 KO mice. The mRNAs included those encoding ICAM-1, osteopontin, and matrix metalloproteinase-2 and -9. At the same time, activity of the matrix crosslinking enzyme, lysyl oxidase, was decreased. When Ang II was infused into ApoE/Ace2 KO mice the aortic dilatation was substantially enhanced compared to control animals. In addition, within 7-days of commencing the infusion, 10 of 12 (83.3%) ApoE/Ace2 KO mice died due to aortic rupture compared to only 2 of 13 control mice (15.4%, P=0.0004). Fatal rupture was observed as early as day-3 after Ang II infusion commencement.

Conclusion: Reduced Ace2 expression, as observed in human aneurysms, promotes vascular inflammation, aortic dilation and rupture in a mouse model of aortic aneurysms. These data support the potential utility of targeting ACE2 in a condition that currently has no medical treatment.

135: EFFECT OF A HIGH FAT DIET AND/OR CHRONIC STRESS ON CARDIOVASCULAR REACTIVITY IN MICE

Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia

Background: Chronic exposure to a high fat diet (HFD) causes hypertension and increased activity of the sympathetic nervous system (SNS). Exposure to chronic aversive stress also influences the SNS and areas in the CNS similar to those activated by a HFD. Thus there is potential for an adverse interaction between a HFD and chronic stress on cardiovascular regulation.

Aim: To investigate the interaction between a HFD and chronic stress on cardiovascular reactivity in conscious mice.

Methods: Male C57Bl6 mice were fed either a normal fat diet (NFD, n=15) or a HFD (n=11) for 10 weeks and then implanted with telemetry probes to record mean arterial pressure (MAP) and heart rate (HR). After recovery mice were divided into non-stressed and chronically stressed groups. The latter involved daily randomized exposure to 60 minutes of restraint and 2 x 30 minutes of dirty cage switch for 3 weeks. The cardiovascular response to acute stress (restraint, dirty cage switch and novel stressors “feeding” and orbital shaker) were examined before and after exposure to chronic stress.

Results: After exposure to HFD, BP was elevated compared to NFD mice (106 versus 97 mmHg, P<0.01), as was HR, but activity was reduced. Exposure to chronic stress reduced BP and HR in both diet groups and reduced BW only in the HFD mice (P<0.05). The pressor and tachycardic response to orbital shaker stress was markedly elevated by chronic stress (+90% and +49%, respectively, P<0.001), as was the response to feeding. HFD attenuated or abolished the chronic stress induced facilitation. By contrast dirty cage switch was reduced and restraint was well maintained after chronic stress. Interestingly, HFD also abolished the habituation induced by chronic stress.

Conclusion: HFD induced hypertension in mice was reduced by exposure to aversive chronic stress. Interestingly, the ability of chronic stress to facilitate pressor responses to novel stressors was abolished by a HFD. Thus there appears to be a mutually beneficial effect on cardiovascular reactivity with the combination of chronic stress and a HFD, presumably involving an interaction within the CNS.
**136: INVESTIGATING THE ROLE OF B CELLS IN THE VASCULAR WALL DURING ANGIOTENSIN II-INDUCED HYPERTENSION IN MICE**

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**Background:** Recent studies by our Laboratory suggest that B cell-deficient mice display a blunted hypertensive response to angiotensin (Ang) II. However, the mechanism(s) by which B cells promote hypertension remain to be determined.

**Aims:** (i) To examine whether Ang II-induced hypertension in mice is associated with the accumulation and/or activation of B cells

**Methods:** Wild-type (C57BL/6J) and B cell activating factor-receptor knockout (BAFF-R\(^{-/-}\)) mice (deficient in B cells) were treated with Ang II (0.7 mg/kg/d, s.c.) or vehicle (0.9% saline) for 14 days. Systolic blood pressure (BP) was monitored via tail cuff and, at day 14, mice were killed, and aortas, serum and spleens were collected.

**Results:** Ang II treatment for 14 days increased systolic BP in wild-type mice from 118±1 mmHg to 155±3 mmHg (P<0.001; n=25).

Background: Despite finding no evidence of B cell accumulation in the aorta, B cells from Ang II-treated wild-type mice appear to be more activated and potential sources of antibodies, chemokines and superoxide, which together may contribute to vascular inflammation and dysfunction during hypertension.

**137: INFLAMMASOME ACTIVITY IS ESSENTIAL FOR DEOXYCORTICOSTERONE ACETATE/SALT-INDUCED HYPERTENSION IN MICE**

Krishnan SM\(^a\), Sobey CG\(^a\), Kemp-Harper B\(^a\), Chan CT\(^a\), Diep H\(^a\), Dowling J\(^a\), Pinal A\(^a\), Mansell A\(^a\), Drummond GR\(^a\)

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**Background:** Inflammasomes are signaling complexes comprised of a NOD-like receptor protein (NLRP), an adapter protein (ASC) and caspase-1. Inflammasomes detect host-derived danger signals, causing activation of caspase-1, which in turn cleaves the cytokines pro-interleukin (IL)-1β and pro-IL-18 into their active, pro-inflammatory forms. Hypertension is associated with chronic renal inflammation, but the role of the inflammasome in this process is not known.

**Aims:** To investigate whether deoxycorticosterone acetate (DOCA)/salt-induced hypertension in mice is associated with increased expression and/or activation of the inflammasome in the kidney, and assess the impact of inhibition of inflammasome activity on blood pressure (BP) and markers of renal inflammation and fibrosis.

**Methods:** Male C57BL6/J (wild type) and ASC\(^{-/-}\) mice were uninephrectomized, implanted with a DOCA pellet (2.4 mg/d, 21 d, s.c.) and had their drinking water replaced with 1% saline (1K/DOCA/salt). Control mice also had a kidney removed but received a placebo pellet and normal drinking water.

**Results:** 1K/DOCA/salt-treated mice had elevated systolic BP (146±4 mmHg) compared to control mice (115±2 mmHg; n=13–16; P<0.05). 1K/DOCA/salt-induced hypertension was associated with increased renal mRNA (fold-change vs control; n=7–9; P<0.05) of inflammasome subunits NLRP3 (2.3±0.2), ASC (2.8±0.6) and pro-caspase-1 (2.6±0.5), and the cytokine, pro-IL-1β (4.0±0.8).

Moreover, protein levels of cleaved (active) caspase-1 and IL-1β were increased by 1.6±0.2- and 2.1±0.3-fold, respectively in kidneys of 1K/DOCA/salt vs. control mice (n=6; P<0.05). ASC\(^{-/-}\) mice, which lack an active inflammasome complex, displayed blunted hypertensive responses to 1K/DOCA/salt-treatment (140±3 mmHg) compared to wild types (155±8 mmHg; n=8–9; P<0.05). ASC\(^{-/-}\) mice were also protected from 1K/DOCA/salt-induced increases in renal levels of mRNAs for inflammatory proteins IL-6, IL-17a, CCL2, ICAM-1 and VCAM-1, and accumulation of collagen.

**Conclusion:** Inflammasome activation, fibrosis and elevated BP in response to 1K/DOCA/salt-treatment are critically dependent on inflammasome activity, highlighting this signaling complex and its cytokine products as potential therapeutic targets to treat hypertension.
138: INFLAMMATORY MECHANISMS IN ABDOMINAL AORTIC ANEURYSM

Channon, Keith M.
University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom

Abdominal Aortic Aneurysm (AAA) is an important disease with high mortality if not detected, and no specific therapies to prevent or slow progression, in order to avoid the need for major surgery. With have investigated novel, cell-specific inflammatory mechanisms in AAA, in order to identify new therapeutic targets.

First, we tested the specific role of the vascular endothelium in AAA pathogenesis. In patients with vascular disease, abnormal endothelial function is a prominent feature, characterised by loss of nitric oxide bioactivity and increased generation of reactive oxygen species (ROS). However, it is not known whether these changes in the endothelium are merely a marker or consequence of disease, or whether endothelial dysfunction and ROS generation are sufficient to drive AAA pathogenesis. We generated mice with transgenic endothelial cell-specific over expression of Nox2, a component of the NADPH oxidase that is increased in vascular diseases. Following infusion of angiotensin II, endothelial Nox2 transgenic mice had a high rate of AAA formation and dissection, which were not observed in wild type controls. Mechanistic studies identified Nox2-induced endothelial cell activation and secretion of cyclophilin A as a key determinant of the susceptibility to AAA, resulting in VSMC activation, inflammatory cell recruitment and MMP secretion in the vascular wall. Thus, the vascular endothelium is a rational therapeutic target in AAA pathogenesis.

Second, we sought to identify new mechanisms in inflammatory cell recruitment in AAA. Using gene array analysis, followed by gene expression studies in human AAA tissue, we identified RGS1, a regulator of G-protein coupled chemokine receptor signalling, as a potential new target in vascular disease pathogenesis. ApoE knockout mice with targeted deletion of RGS1 showed striking protection from angiotensin II-induced AAA formation and rupture, despite increased chemotactic activity in RGS1 knockout macrophages. Mechanistic studies revealed a role for RGS1 in inflammatory cell accumulation in the vascular wall, demonstrating that inflammatory cell retention, not just recruitment, is a key determinant of susceptibility to AAA. These new cell-specific mechanisms illustrate the important of identifying rational molecular targets for future therapies in AAA, and will be addressed in clinical studies.

139: POTENTIAL MECHANISMS AND THERAPIES FOR ABDOMINAL AORTIC ANEURYSM: TRANSLATING FINDINGS FROM MICE TO PATIENTS

Moxon, Joseph V
Queensland Research Centre for Peripheral Vascular Disease, Australia

An incomplete understanding of the mechanisms driving the formation, progression and rupture of abdominal aortic aneurysms (AAA) is reflected by significant shortfalls in patient care. Investigating the molecular biology of biopsies taken from the aortic wall of AAA patients has the potential to identify novel therapeutic targets and diagnostic/prognostic markers. However, human AAA biopsies can only be collected from the occasional patient undergoing open surgery for a large AAA. Moreover, obtaining appropriate control tissues from non-aneurysmal human donors can be challenging. Thus, infrequent access to experimental tissues can present a significant bottleneck to AAA research. To overcome this, several animal models that mimic key features of human AAA have been developed. Using these models, significant insights into the molecular processes underpinning AAA pathogenesis have been gained. However, the potential for these findings to be translated to the clinical situation and improve patient management is not always clear.

Researchers at the Queensland Research Centre for Peripheral Vascular Disease routinely employ the angiotensin-II infused hyperlipidaemic mouse model to investigate AAA pathology. Findings of our research have identified key similarities and differences in the mechanisms driving AAA in experimental mice and human patients. The current talk will outline the experiences of the Queensland Research Centre for Peripheral Vascular Disease in the use of the angiotensin-II infused mouse model of AAA, and discuss strategies employed to translate findings from our mouse-based research to the clinical situation.

140: THROMBOSPONDIN-1 DEFICIENCY PROMOTES MALADAPTIVE EXTRACELLULAR MATRIX REMODELING IN ABDOMINAL AORTIC ANEURYSM

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Thrombospondin (TSP)-1 is a member of ‘counter-adhesive matricellular’ protein family, grouped mainly based on their functional similarity. The aim of the present study was to assess the role of TSP-1 in the formation of abdominal aortic aneurysm (AAA) formation. Serum TSP-1 concentration in male AAA patients (n=276) were measured by ELISA. The AAA patients were monitored by ultrasound scans for a median of 5.5 years. AAA growth was negatively
correlated with serum TSP-1 concentration (P=0.033). After adjusting for cardiovascular risk factors, men with TSP-1 in the highest quartile were less likely to show growth greater than median during follow-up (OR 0.4; 95% CI, 0.19-0.84, P=0.016). To further assess the role of TSP-1 in AAA initiation and progression, we assessed AAA formation in 12 week old apolipoprotein E deficient (ApoE-/Tsp1+/+, n=20) and ApoE-/Tsp1-/- (n=20) male mice receiving Angiotensin II (AngII). After 28 days of AngII infusion, the Tsp1-/- mice showed larger aneurysms on in vivo ultrasound (P<0.05), and ex vivo morphometry (P<0.001) than ApoE-/ mice. Plasma MCP-1 (P<0.05) and IL-6 (P<0.05) concentrations in the Tsp1-/- mice were significantly higher than ApoE-/ along with elevated suprarenal aortic matrix metalloproteinase (MMP)-9 gene expression (P<0.05). Collectively, findings from this study suggest that TSP-1 deficiency promotes maladaptive remodelling of the extracellular matrix leading to accelerated AAA progression.
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AAS 01: RS4149337 AND RS2066881 SINGLE NUCLEOTIDE POLYMORPHISMS IN ABCA1 GENE IS ASSOCIATED WITH LOW SERUM HIGH-DENSITY LIPOPROTEIN CONCENTRATION

Ahmad WNHBW, Mohksin NAM, Sakri FH, Peng HB, Rahman T, Nasir NM, Razak SA, Yasin MM, Nawawi HM
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Background: Coronary artery disease (CAD) is the major cause of death worldwide. High-density lipoprotein cholesterol (HDL-c) plays a vital role in reverse cholesterol transport and its concentration is inversely correlated with CAD risk. ATP-binding Cassette Transporter A1 (ABCA1) is the main transport protein in reverse cholesterol transport and its gene variants have been linked to low HDL-c concentration. rs4149337 and rs2066881 single nucleotide polymorphisms (SNPs) have been found in 40% of East Asian population, also its association with low serum HDL-c concentration is unclear.

Aim: To identify and characterize ABCA1 SNPs among subjects with low HDL-c concentration compared to normal controls.

Methods: Seventy subjects (41 females, HDL-c ≤0.70 mmol/L and 29 males, HDL-c ≤0.60 mmol/L, based on the HDL-c concentration cut-off of the 2.5% normal local population distribution) and 140 age-, gender-, ethnic-, diabetes- and smoking-matched controls (87 females, HDL-c ≥ 1.3 mmol/L and 53 males, HDL-c ≥ 1.0mmol/L) were recruited for this study. Whole blood samples were collected for DNA extraction. Target region of ABCA1 gene amplification was carried out by polymerase chain reaction. Amplified DNA fragments were sequenced. Confirmation of the SNPs was analysed using BioEdit.

Results: DNA sequencing of ABCA1 gene revealed six SNPs. However, two SNPs (rs4149337 and rs2066881) were found to be significantly different in both genotype and allele frequencies in low HDL-c subjects compared to controls (p-value <0.05).

Conclusions: rs4149337 and rs2066881 SNPs in ABCA1 gene appear to be associated with low HDL-c concentration. Further studies with larger sample size are required to verify these associations, which could be responsible for enhanced CAD prevalence in low HDL-c subjects, especially among those without other coronary risk factors.

AAS 02: VALIDATION AND USE OF A TRIGLYCERIDE METER IN LATE PREGNANCY

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Background and Aims: Elevated maternal triglycerides have been associated with adverse pregnancy outcomes including an increased risk of preeclampsia and macrosomia. A practical triglyceride meter would allow examination of maternal postprandial triglycerides.

Methods: A non-fasting venous and capillary (using the Roche Accutrend® Plus meter) triglyceride measurements were taken in 40 participants at a mean of 36 weeks gestation. Following this validation phase, the meter was trialled for home triglyceride monitoring: 4 times a day (fasting and two hours post each meal) for 6 days in late gestation in 12 women with and without gestational diabetes mellitus (at more than 36 weeks gestation).

Results: Venous and capillary methods were highly correlated (r = 0.89, P <0.0001), and the distributions were similar (mean difference 0.01 mmol/L (SD 0.47)), t=0.18, P =0.86). Passing Bablok equation was: y = -0.01 + 0.98x [95% CI intercept -0.51 – 0.38; 95% CI for the slope 0.85 – 1.15). The estimated bias was 0.01 mmol/L (95%CI -0.93 – 0.91)). To date, 12 women have trialled the meter at home. Median triglycerides were: fasting 2.89 mmol/L (95% CI 2.77 – 3.46), postprandial 3.39 mmol/L (3.27 – 3.70).

Conclusions: This study demonstrated the triglyceride meter provides results correlated strongly with the reference method with low bias when used in late pregnancy. In home use, median maternal triglycerides did vary greatly over the day. Further exploration of the practicalities of the use of this meter is needed prior to embarking on a larger scale trial.

AAS 03: HDL IMPROVES GLUCOSE HOMEOSTASIS AND REDUCES ATHEROSCLEROSIS IN TRAIL-/-/APOE-/-/ MICE

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Background and Aims: Diabetes is associated with increased risk of cardiovascular disease (CVD) including atherosclerosis and myocardial infarction. Diabetic patients have elevated plasma low density lipoprotein (LDL) and triglycerides, and low levels of high density lipoprotein (HDL). Recently, we demonstrated that TNF-α-related apoptosis-inducing ligand (TRAIL) deficiency in ApoE-/- mice in response to a ‘western’ diet, displayed accelerated atherosclerosis and promoted features of diabetes typical of human disease. Apolipoprotein A-I (apoA-I), the main component of HDL, can increase insulin secretion in pancreatic β-cells, suggesting that interventions which raise HDL levels may be beneficial in treating not only diabetes, but also lowering cholesterol in CVD. Based on these findings we sought to determine the effects of
reconstituted HDL (rHDL) in TRAIL-/-ApoE-/- mice. 

Methods and Results: TRAIL-/-ApoE-/- mice on a high fat ‘western’ diet for 12 w received 3 weekly infusions of either PBS (vehicle) or rHDL (containing apoA-I (20mg/kg) and 1-palmitoyl-2-linoleoyl phosphatidylcholine) 2 weeks prior to sacrifice. While no change in weight gain was observed with treatment, administration of rHDL significantly reduced total plasma cholesterol, triglyceride and glucose levels. Notably, rHDL treated TRAIL-/-ApoE-/- mice had significantly reduced plaque size in the brachiocephalic artery. Furthermore, rHDL treatment improved glucose clearance in response to insulin and glucose tolerance tests. Immunohistological analysis of pancreatic islets revealed an increase in insulin expression/production and a reduction in macrophage infiltration. TRAIL-/-ApoE-/- mice treated with rHDL showed elevated levels of CD11c-expressing splenic cells, suggesting increased numbers of dendritic cells.

Conclusions: This is the first demonstration where rHDL improves atherosclerosis and rescues features of diabetes which are dependent on TRAIL. These findings support the therapeutic potential of rHDL in the treatment of diabetes associated CVD.

AAS 04: DESCRIBING THE EFFICACY OF HDL IN EARLY ATHEROSCLEROSIS VIA MATHEMATICAL MODELLING

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The University of Sydney, Sydney, Australia

Background and aims: HDL raising therapies have not had the desired results in drug trials. These trials either fail to show that increasing HDL-C reduces heart attacks or strokes in patients who are on LDL-C therapies or they have been discontinued as the trial drug had significant adverse side effects. We introduce a qualitative mathematical model to explore early atherosclerosis and analyse plaque development under different physiological conditions. We extend this model to the effects of HDL therapy.

Methods: Atherosclerosis is initiated by a specific sequence of events that follows endothelial failure. We constructed a model that encapsulates the primary interactions between modified LDL, monocytes and macrophages, chemoattracants, endothelial-stimulating cytokines and foam cells immediately following endothelial failure. We consider the dynamics of the interactions between these species both at the endothelium and inside the intima. We include three primary atheroprotective actions of HDL: reverse cholesterol transport; LDL modification inhibition; and decreased monocyte adhesion. We computed outcomes for this model for different rates of influx of functional HDL into the intima.

Results: Using the model, we can visualize the changing density and distribution of macrophages and foam cells in the early plaque as a function of time. The model shows qualitatively how atherosclerosis is initiated and how foam cells accumulate after the initial inflammatory period. The model predicts that increases in the influx of functional HDL into an early plaque causes the simulated plaque to cease to grow and perhaps regress. However, if HDL influx is increased by the same amount but at a later time in plaque development, then the plaque continues to grow. We compute our results on a two-dimensional cross section of the artery, and include the distortion of the blood vessel wall by plaque development.

Conclusions: The computed results of the model suggest that the stage of plaque development may be important as well as increasing functional HDL influx in determining whether or not a plaque regresses and that the dynamics involved in the early stages of atherosclerosis have an influence on the efficacy of HDL action.

AAS 05: APOAI MIMETIC PEPTIDES AND ATHEROSCLEROSIS

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Background: Atherosclerosis is a multifaceted arterial disorder that underlies multiple cardiovascular events. A promising approach for treatment of atherosclerosis is boosting levels and/or functionality of HDL through administration of HDL mimetics, including apoA-I mimetic peptides. In this study, we addressed a question of contribution of specific anti-atherogenic properties of apoA-I mimetic peptides to their overall anti-atherosclerotic activity. We focused on the peptides that have shown in vitro a high efficiency in reducing endothelial cell activation and LDL oxidation (5A-C1), monocyte activation and cholesterol efflux (ELK-2A2K2E) and a combined treatment with these peptides using the mouse model of atherosclerosis.

Methods: Fluorescently labelled peptides were injected into ApoE mice and blood was collected over 48 hour period to determine peptide kinetics. ApoE deficient mice were placed on high fat diet and treated with peptides for 4 and 12 weeks. Collected serum was analysed for lipid contents. Atherosclerosis progression and plaque composition was studied on en face aorta and cross sections of aortic sinus.

Results: Peptides demonstrated only minor differences in their kinetics with majority of peptide being removed from circulation after 12 hours with minor quantities detectable at 48 hours. Peptides showed different affinity to lipoprotein fractions with the majority of ELK-2A2K2E found in HDL, while 5A-C1 was mainly found in LDL. All peptides reduced the lesion burden to a similar level by reducing lesion growth and/or by inhibiting lesion initiation. Despite similar effects on the lesion size, the peptides have different effect on lipid and macrophage accumulation in the plaque. Combination treatment resulted in reduced total serum cholesterol, triglycerides, while the individual peptides had no effect on plasma lipoprotein profile.

Conclusion: ApoA1 mimetic peptide administration reduce atherosclerosis burden in mouse model of atherosclerosis. While the overall effect of the treatment was similar for all tested apoA1 mimetic peptides, they appear to act by different mechanisms.
Familial Hypercholesterolaemia (FH) is a genetic condition which produces a clinically recognizable pattern with elevated low-density lipoprotein cholesterol (LDLC) levels leading to accelerated atherosclerosis and increased risk of premature cardiovascular disease, coronary artery disease and stroke. A number of different mutations are known to be associated with FH, the most common of which affect the low-density lipoprotein receptor (LDLR) gene and can disrupt various mechanisms such as receptor synthesis or the ability of the receptor to bind to LDLC. A high degree of phenotypic variability exists among patients diagnosed with FH, but previous evidence suggests that FH patients with a recognized mutation have higher total cholesterol (TC) and LDLC than mutation negative FH patients, and possibly a more severe clinical outcome.

The aim of this study was to establish whether patients with mutation positive FH have more severe cardiovascular outcomes than patients with mutation negative FH.

A dataset was constructed of patients who underwent FH screening for identifiable mutations on the basis of clinically defined FH, or pre-treatment total cholesterol levels ≥ 8mmol/L. The dataset included extensive information on patient demographics, family history, mutation status, the occurrence of cardiovascular disease or mortality, and clinical and biochemical features measured at the index Lipid Disorders Clinic visit. Categorical variables were compared between groups using Chi-square or Fisher’s exact tests, and differences in continuous biochemical and clinical parameters among the groups were tested using analysis of variance and Fisher’s least significant difference test as appropriate.

Preliminary results showed significantly higher mean TC and LDLC levels in mutation positive FH patients than in mutation negative FH patients at the time of their index visit to the Lipid Disorders Clinic. No significant differences in the presence of cardiovascular disease at the time of the index visit, or mortality, were observed between mutation positive and mutation negative FH patients.

Data is now available on 1700 patients, and results will be updated accordingly.

Although significant differences in cardiovascular outcome have yet to be determined, mutation positive FH patients present with higher mean TC and LDLC levels than their mutation negative counterparts. Further investigation of the relationship between mutation status and the occurrence of cardiovascular events among FH patients will allow greater understanding of the disorder.

AAS 06: FAMILIAL HYPERCHOLESTEROLAEMIA AND CARDIOVASCULAR OUTCOMES

Francis MM, Young JM, GeorgePM


AAS 07: NOX/P47PHOX ACTIVATION AND OXIDATIVE STRESS IN ACUTE MACROPHAGE NECROSIS

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Introduction: The mechanism causing oxLDL to be cytotoxic to human macrophages has been recently questioned by our finding that the oxysterol 7-ketocholesterol is not cytotoxic when inside LDL particles1. We found that both Cu-oxLDL2 and HOCl-oxLDL triggers an oxidative stress dependant necrosis in human macrophages, which is not related to the oxysterol levels. OxLDL initiation of macrophage necrosis does not require uptake of significant amounts of oxLDL, suggesting the necrosis is induced by the cells own response to the particles

Aims: Determine role of intracellular oxidants and NADPH oxidase (NOX) activation in oxLDL induced macrophage necrosis.

Methods: U937 cells, human monocytes and macrophages were cultured in serum supplemented RPMI1640 media. LDL prepared by ultracentrifugation of human plasma, was oxidised with Cu++ or HOCl. Cell necrosis and ROS generation was measured by flow cytometry; GSH by HPLC; enzyme activity by spectrophotometric assays; and p47phox levels by western blot analysis.

Results and Discussion: OxLDL caused a rapid rise in intracellular oxidants measured as an increase in DHE fluorescence, which peaked after 6 hours. Intracellular levels of the NOX activator p47phox increased significant while levels of GSH, enzymatic GAPDH activity, lactate release and mitochondrial potential decreased.

Conclusion: In U937 cells the NOX/p47phox inhibitor apocynin significant reduced the level of oxidants, GSH loss and cell death. This suggests oxLDL activation of NOX may promote macrophage cell death and the growth of the necrotic core of atherosclerotic plaques.

AAS 08: STATUS OF OXIDATIVE STRESS BIOMARKERS IN FAMILIAL HYPERCHOLESTEROLEMIA

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Purposes of Study: Oxidative stress is the initial underlying cause of atherosclerosis. Familial Hypercholesterolemia (FH) patients are known to be at risk in developing atherosclerosis. Thus, this study aims to compare the oxidative stress biomarkers level between FH patients and normal control, besides to its correlation and association with LDL cholesterol (LDL-c).

Methods: 121 FH subjects diagnosed by Simon Broome Criteria, and 172 age, gender, ethnic, smoking, BMI, Diabetes status matched controls were recruited for this study. The blood samples were collected into a plain tube which was immediately centrifuged to separate the serum and stored at-20°C until analysis. Measurement of ox-LDL was performed using a commercially available sandwich enzyme-linked immunosorbent assays (ELISA), (Mercodia,
Results: There was a significant difference in ox-LDL levels between FH patients and controls (Mean ± SEM: 63.0 ± 6.5 vs 25.5 ± 1.2; p<0.001). F2-Isoprostane level was significantly higher in FH patients as compared to controls (Mean ± SEM: 749.7 ± 74.0 vs 354.2 ± 18.1; p<0.001). MDA level of FH patients were also significantly elevated in comparison with controls (Mean ± SEM: 342.4 ± 46.7 vs 162.7 ± 13.5 nmol/g, p<0.0001). Pearson’s correlation analysis showed positive correlation of LDL-c concentration with all oxidative stress biomarkers, ox-LDL (p<0.001, r=0.597), F2-Isoprostane (p<0.001, r=0.489), and MDA (p<0.0001, r=0.362). Chi square analysis of LDL-c concentration and quartiles of oxidative stress biomarkers between FH patients and controls was significantly associated with the highest quartiles of ox-LDL, F2-Isoprostane and MDA (p<0.001).

Conclusion: FH patients have higher oxidative stress level compared to normal control which suggests that patients with FH are at greater risk of developing atherosclerosis. The positive association and correlation between LDL-c levels and these oxidative stress biomarkers could indicate as a potential predictor of atherosclerosis manifestation.

AAS 9: APOLIPOPROTEIN A-I MIMETIC PEPTIDES ARE A POTENTIAL TREATMENT FOR INSULIN RESISTANCE

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Background and Aim: Apolipoprotein-AI (apo-AI) is the major apolipoprotein found in high-density lipoprotein particles (HDLs). Recently, we showed that high fat diet-fed C57Bl/6 mice treated with full-length apo-AI for up to 4-weeks improved insulin resistance. While this data provides compelling proof of concept data, intact apo-A-I is not suitable as a therapy option due to the time and cost associated with its production and administration. The aim of the present study was to test whether apo-A-I mimetic peptide treatment emulated the effects of full-length apo-A-I to improve insulin resistance.

Method and Results: Insulin resistant C57Bl/6 mice were generated by high-fat feeding (HFD) for 16-weeks. Mice treated with apo-A-I mimetic 5F peptide synthesised from L-amino acids (L-5F; administered by twice weekly intraperitoneal injections) or treated with apo-A-I mimetic peptide 4-F synthesised from D-amino acids (D-4F; administered via drinking water) showed: (i) improved glucose tolerance and insulin sensitivity that was associated with decreased hepatic inflammation (TNFa, IL6, IL-1β and IFN-γ); (ii) suppression of hepatic mRNA expression of gluconeogenesis-associated genes (PEPCK and G6Pase) and lipogenic-associated genes (SREBP1c and ChREBP) and; (iii) reduced hepatic macrophage infiltration.

Conclusions: We conclude that both the apo-A-I mimetic peptides, L5-F and D4-F, improve insulin resistance in HFD-fed C57Bl/6 mice. This effect is associated with reduced expression of inflammatory markers in the liver, reduced infiltration of macrophages and altered gene expression of genes associated with gluconeogenesis and lipid synthesis suggesting that glucose and lipid synthesis is suppressed. Together, these findings suggest that apo-A-I mimetic peptides could be considered as a new therapeutic option to reduce hepatic inflammation that contributes to the development of overnutrition-induced insulin resistance. This is important consideration as rates of insulin resistance are increasing in society and insulin resistance is a risk factor for atherosclerosis.

AAS 10: ASSOCIATION ANALYSIS OF APOLIPOPROTEIN B AND VERY LOW-DENSITY LIPOPROTEIN WITH HYPERURICEMIA AND GOUT

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Background and Aims: Gout results from an innate immune response to monosodium urate (MSU) crystals deposited in joints. Increased very low-density lipoprotein (VLDL) has been associated with gout. Apolipoprotein B (apoB), present on VLDL, regulates neutrophil response to MSU crystals. ApoB has been positively associated with gout and the APOB mRNA-editing gene, A1CF, is associated with urate levels. However the relationship of ApoB and VLDL with gout in the presence of hyperuricemia has not previously been tested. Therefore we tested the association of VLDL and apoB with gout in the presence of hyperuricemia (HJ).

Methods: New Zealand European (n = 90) and Māori and Pacific Island (Polynesian) (n = 90) male gout case and control sample sets were divided into normouricemia (NU: serum urate <0.41mmol/L), asymptomatic hyperuricemia (HU: serum urate ≥0.41mmol/L) and gout groups. Gout was classified using the 1997 American Rheumatism Association criteria. Size exclusion chromatography and enzyme-linked immunosorbant assay were used to measure VLDL and apoB. Multivariate linear regression was used to assess the risk of gout and HU per unit change in VLDL and apoB.

Results: Increased levels of VLDL triglycerides (Tg) were observed in the gout sample set compared to NU and HU in European (P=1×10-4 and 2×10-3, respectively) and Polynesian subjects (P=0.042 and 0.019, respectively). This increase was driven by overproduction of VLDL particles in the European subjects and by the Tg-enrichment of existing VLDL particles in the Polynesian subjects. Each mmol/L increase in VLDL Tg was significantly associated with gout in the presence of HU in Europeans, with a similar trend in Polynesians (OR=6.82, P=0.017 and 2.85, P=0.066, respectively). Each μmol/L increase in apoB was associated with a decreased risk of HU (OR=0.47; P=0.046) and, conversely, with increased risk of gout in the presence of HU (OR=4.79; P=0.005: Table 1) in combined sample set.

Conclusion: Increased VLDL Tg is associated with the risk of gout in the presence of HU. If genetic approaches...
indicate evidence for causality of VLDL in gout, this would provide further justification for clinical trials examining the effects of fibrates as a treatment option in gout.

AAS 11: MECHANISMS OF CYCLOSPORIN A-INDUCED DYSLIPIDEMIA

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Background: Cyclosporin A (CsA) is an immunosuppressant drug commonly used in organ transplant recipients and auto-immune disorders. The long-term treatment with CsA is associated with hyperlipidemia and an increased risk of cardiovascular disease. The mechanism(s) by which CsA causes hyperlipidemia is unknown but are generally thought to be mediated via the Low Density Lipoprotein Receptor (LDLr). However, the data supporting a role for LDLr are inconclusive. To determine whether the LDLr plays a role in CsA-induced hyperlipidemia we examined the effect of CsA in LDLr knockout (LDLr\textsuperscript{-/-}) and wild type C57Bl6/J mice.

Methods: Female mice fed a chow diet were treated with 20 mg/kg/day CsA administered subcutaneously by implantation of Alzet osmotic pumps for four weeks. Liver and kidneys were assessed for toxicity by histology. Lipoprotein fractions were separated by FPLC and lipid levels in plasma and FPLC fractions were measured using commercial enzymatic kits. Specific lipid species were determined by LC-MS/MS. Effect of CsA on hepatic genes and proteins involved in lipid metabolism were investigated by real time PCR and Western Blot analysis. Hepatic VLDL production rates were determined by measuring plasma triglyceride levels after intravenous injection of Triton WR1339 to stop lipolysis and lipoprotein lipase (LPL) activity was determined before and after intravenous injection with heparin using a commercially available kit.

Results: Mice tolerated CsA treatment well and no liver or kidney toxicity was observed. Total plasma cholesterol and triglyceride levels were increased 2-fold and 1.6-fold respectively in CsA-treated LDLr\textsuperscript{-/-} mice, which was associated with a strong increase in plasma VLDL and LDL levels and no changes in plasma HDL levels. No effect on plasma lipids was observed in the C57Bl6/J mice. Analysis of specific lipid species suggested increased VLDL and LDL particle number. In addition small changes in some of the minor lipid species contained within VLDL and LDL were observed. CsA did not affect hepatic VLDL production or secretion but did inhibit plasma LPL activity.

Conclusion: In conclusion, CsA can induce hyperlipidemia independently of the LDLr. This is not mediated via increased hepatic VLDL synthesis or secretion. Inhibition of plasma LPL activity by CsA is likely to contribute to CsA-induced hyperlipidemia. Further work will determine whether CsA also affects lipoprotein clearance in these mice. These data also suggest the possibility that the hyperlipidemic effects of CsA may be more evident in patients with LDLr dysfunction.

AAS 12: PHARMACOLOGICAL INHIBITION OF DYNAMIN II REDUCES CONSTITUTIVE PROTEIN SECRETION FROM PRIMARY HUMAN MACROPHAGES

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Background: Macrophages play an important role in the initiation and progression of atherosclerotic plaque development. Dynamins are fission proteins which endocytic and exocytic membrane events and are pharmacological therapeutic targets. These studies investigate whether dynamin II regulates constitutive protein secretion from primary human macrophages.

Methods: Primary human monocytes were isolated from buffy coats obtained from the Red Cross Blood bank. Cells were differentiated into macrophages and exposed to various classes of dynamin inhibitors. Cellular and secreted proteins were determined by Western blotting. Degradation and secretion rates were determined using [35S]methionine-pulse chase labeling. Processing of apoE was analysed by 2D gel electrophoresis. Intracellular localization and microtubule effects were determined using confocal microscopy on fixed cells. Live cell imaging was performed after transfection with apoE-GFP on a Zeiss LSM confocal microscope.

Results: Inhibitors that target recruitment of dynamin to membranes (MiTMABs) or directly target the GTPase domain (Dyngo\textsuperscript{TM} or Dynole\textsuperscript{TM} series), dose- and time- dependently reduced the secretion of apoE. SiRNA oligo’s targeting all isoforms of dynamin II confirmed the involvement of dynamin II in apoE secretion. Inhibition of secretion was not mediated via effects on mRNA or protein synthesis. 2D-gel electrophoresis showed normal post translation glycosylation indicating that transport inhibition occurred after apoE was processed in the Golgi. Live cell imaging showed that inhibited secretion was associated with reduced post-Golgi movement of apoE-GFP-containing vesicles. The effect was not restricted to macrophages, and was not mediated by the effects of the inhibitors on microtubules. Inhibition of dynamin also altered the constitutive secretion of other proteins, decreasing the secretion of fibronectin, matrix metalloproteinase 9, Chitinase-3-like protein 1 and lysozyme but unexpectedly increasing the secretion of the inflammatory mediator cyclophilin A.

Conclusion: We conclude that pharmacological inhibitors of dynamin II modulate the constitutive secretion from macrophages as a class effect, and that their capacity to modulate protein secretion may affect the biology of diseases involving macrophage infiltration including atherosclerosis.
Formation of functional collateral circulation around blocked arteries is an important process in amending adverse outcomes after acute coronary occlusion events. Inadequate capillary growth during pressure overloads impairs myocardial perfusion, often contributing to the progression of coronary heart disease. Considered to be the critical rate limiting step in physiological angiogenesis, the binding of VEGF (Vascular endothelial growth factor) to VEGFR-1 (Vascular endothelial growth factor receptor) is essential for the growth and repair of arteries. However, an alternatively spliced soluble form of VEGFR-1 (sFlt-1) has been shown to inhibit VEGF activity. sFlt-1 acts as a decoy, binding free extracellular VEGF with high affinity, thus preventing it from binding VEGFR-1. As a result, the primary pathway of angiogenesis does not occur. Rapid progression of coronary heart disease is often clinically silent, without signs or symptoms. For this reason the ability of markers to monitor progression is a powerful tool for predicting cardiovascular risk. Recent findings of this study show, that in 306 patients, an above median sFlt-1 was a strong predictor of admission for HF (hazard ratio = 1.84, p=0.035), independent of age and gender. This suggests that sFlt-1 may be a useful prognostic marker for coronary heart disease with potential to aid prediction of future ischemic events in previously diagnosed patients. In support of these findings, levels of sFlt-1 measured in plasma taken from patients, prior to undergoing carotid endarterectomy procedures (n=27), where significantly raised in comparison to age and gender matched healthy controls (P<0.001). Levels of sFlt-1 in patient and control groups were shown to be independent of both age and gender. This indicates that, independently of both age and gender , the measurement of sFlt1 as a biomarker of vascular disease may have great potential for future diagnostic use in a clinical setting.

**AAS 14: RIBOSE-CYSTEINE INCREASES GLUTATHIONE-BASED ANTIOXIDANT STATUS AND REDUCES LDL VIA A STATIN-LIKE EFFECT IN MICE**

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**Background and aims:** Ribose-cysteine is a cysteine analogue designed to increase the synthesis of glutathione (GSH). GSH is a cofactor for the glutathione peroxidase (GPx) enzyme that catalyses the reduction of lipid peroxides. A low GPx activity and increased oxidised lipids are associated with the development of cardiovascular disease (CVD). We aimed to investigate the effect of ribose-cysteine on GSH, GPx, lipid oxidation products and plasma lipids in vivo.

**Methods:** Human lipoprotein(a) [Lp(a)] transgenic mice (n=9), which display a human-like lipid profile, were treated with 4 mg/day ribose-cysteine (0.16 g/kg body weight) for 8 weeks. Blood, livers and aortae were harvested from treated and untreated controls and GSH concentrations, GPx activity, thiobarbituric acid reactive substances (TBARS), F2-isoprostanes and plasma lipid concentrations were measured. The amount of apolipoprotein B (apoB), low density lipoprotein receptor (LDLR) and 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase mRNA transcripts in the livers was assessed by quantitative PCR. The concentration of apoB and apo(a) protein in the liver was quantified by ELISA and the amount of LDLR and HMGCoA reductase protein was assessed by western blots.

**Results:** Ribose-cysteine treatment significantly increased GSH concentrations in the liver and plasma (1.3 and 2.5 fold respectively, P<0.05) and also increased GPx activity in the liver (1.7 fold, P<0.01) and erythrocytes (3.5 fold, P<0.05). TBARS concentrations in the liver, plasma and aortae were significantly reduced (P<0.01, P<0.0005 and P<0.01, respectively) as was F2-isoprostane levels in the liver (P<0.0005). Ribose-cysteine treatment was associated with significant decreases in LDL, Lp(a) and apoB concentrations (P<0.05, P<0.01 and P<0.05) but did not significantly affect HDL cholesterol or triglyceride concentrations. Analysis of mRNA transcripts in the liver showed the expression of LDLR to be increased 3.5 fold (P<0.0001) in ribose-cysteine treated mice with no changes in apoB or HMGCoA. A similar result was seen at the protein level with LDLR increased 2.7 fold (P<0.0005).

**Conclusions:** Ribose-cysteine exerts an antioxidant effect by increasing GSH-based antioxidant status and lowering the level of oxidised lipids. It also has LDL and Lp(a)-lowering properties which are associated with a statin-like effect. As elevated LDL, Lp(a) and oxidised lipids are associated with the development of CVD, ribose-cysteine might be an ideal supplementary intervention to increase protection against CVD.

**AAS 15: PLASMA LIPID PROFILING TO PREDICT CARDIOVASCULAR EVENTS IN TYPE 2 DIABETES**


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**Background:** 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 (MI),
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 CVE in patients with T2D. Plasma lipids (310 species) 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=698) and T2D patients who did not have a CVE (n=3081). Weighted Cox regression was used to identify and rank lipid species associated with future CVE. Multivariate models combining traditional risk factors alone or with the top ranked lipid species within a bootstrapping framework were used to evaluate the ability of plasma lipids to improve upon traditional risk factors to discriminate and reclassify five-year risk. C-statistics (AUC) and net reclassification improvement (NRI) were calculated.

**Results:** We observed significant associations between 47 lipids and CVE (p<0.05, corrected for multiple comparisons 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. Compared to the base model containing 14 traditional risk factors, the addition of 20 lipid species resulted in an increase in AUC of 0.027 to 0.721 (95% CI, 0.687-0.745), a NRI of 8.1% (95% CI, 1.6% – 17.9%) based on a categorical model of <10, 10–15, and >15% risk) and a continuous NRI of 36.0% (95% CI, 22.9% – 49.2%). Models specific for future MI (AUC 0.754 (0.721-0.788), categorical NRI 20.3% (5.2% – 34.9%)) and stroke (AUC 0.725 (0.669 – 0.767), categorical NRI 13.5% (-0.3% – 31.8%) also showed improved performance.

**Conclusion:** The strong associations between plasma lipids and future CVE provide insight into the role of lipids in the pathogenesis of CVD and identify potential therapeutic targets. The improvement in the prediction of CVE, above traditional risk factors, demonstrates the potential of plasma lipids as biomarkers for CVD risk stratification in T2D.

**AAS 16: THYMOQUINONE AND NIGELLA SATIVA INHIBIT IN VITRO LIPOPOLYSACCHARIDE–INDUCED ADHESION OF MONOCYTES TO HUMAN CORONARY ARTERY ENDOTHELIAL CELLS**

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**Background:** Monocyte adhesion to human aortic endothelial cells is one of the early events in the development of atherosclerosis. Human Coronary Artery Endothelial Cells (HCAECs) were used to investigate the role of thymoquinone (TQ) and Nigella sativa oil (NSO) in human monocyte adhesion to HCAECs in vitro. TQ, one of the bioactive compounds in Nigella sativa (black seed), has been postulated to have possible anti-atherosclerotic properties. However, its effects together with NSO on adhesion of human monocyte to HCAECs in vitro remain scarce.

**Aims:** To determine the inhibitory effect of TQ and NSO on lipopolysaccharide (LPS)-induced production of Soluble Intercellular Adhesion Molecule 1 (sICAM-1) and adhesion of monocytes to HCAECs.

**Methods:** HCAECs were stimulated for 24 hours with 1µg/ml of LPS, with different concentrations of TQ (4.5, 9, 18, 36µM) and NSO (55, 110, 220, 440µg/ml). Rose Bengal Assay was used and measured with spectrophotometer at 570nm. Positive and negative controls were performed in parallel. Results were expressed as fold changes from unstimulated cells. sICAM-1 gene and protein expressions were measured using QuantiGene 2.0 Multiplex Assay and Procarta Assay respectively.

**Results:** HCAECs pretreated with 4.5, 9, 18, 36µM TQ for 24 hours showed lower adherence for monocytes compared to non-treated HCAECs (Mean±SEM/[% inhibition]; 1.18±3.3×10⁻³/8.33%), 1.17±17.6×10⁻³/8.33%, 1.22±3.3×10⁻³/4.43%), 0.89±3.3×10⁻³/30.73% versus 1.28±11.5×10⁻³/p<0.05 and 0.001 respectively). HCAECs pretreated with 55, 110, 220, 440µg/ml NSO for 24 hours also showed lower adherence for monocytes compared to non-treated HCAECs (Mean±SEM/[% inhibition]; 1.33±3.3×10⁻³/9.75%, 1.38±0.1/6.12%, 1.35±19.2×10⁻³/8.39%, 1.33±8.9×10⁻³/9.3% versus 1.47±5.7×10⁻³; p< 0.002). TQ and NSO significantly reduced sICAM-1 gene and protein expressions: TQ [gene: at 36µM (p<0.001) protein: at 4.5, 9, 18, and 36µM (p<0.001)] and NS [gene: at 55, 110, 220, 440µg/ml (p<0.05 and 0.001 respectively) protein: at 55, 110, 220, 440µg/ml (p<0.01 and 0.001 respectively)].

**Conclusion:** TQ and NSO supplementation in vitro inhibit monocytes adherence to HCAECs via downregulation of sICAM-1 expression.

**AAS 17: CHRONOBIOLICAL FACTORS INFLUENCING THE DEVELOPMENT OF ACUTE MYOCARDIAL INFARCTION**

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**Purpose:** To identify the peculiarities of MI, the severity of its course and outcome, as well as, the hemostasis system under conditions of sharp continental climate.

**Materials and methods:** 420 patients with AMI, aged 35-70 years were studied by standard techniques based on uniform diagnostic criteria. All the patients were divided into 2 Groups: the Group 1, consisted of patients without ST elevation and the Group 2, consisted of patients with ST elevation.

**Results:** Primary MI was registered in 73.8% of the cases and recurrent MI was registered in 26.2% of the cases; complicated myocardial infarction was occurred in 30.4% of the cases. Primary MI with complicated course comprised 28.1%, where recurrent MI complicated course comprised 37.2%. In Group 1, complication comprised 27.3%,
where in Group 2, it was 36.7%. The total number of deaths was registered in 65 cases (15.5%). In primary AMI 63.1% of patients died, where it was 36.9% in recurrent AMI. The number of deaths in patients of Group 1 and Group 2 was not differed: 42.9% and 57.1%, respectively. In complicated MI, deathswere 73.8% in summer, and 26.2% in winter. In the summer, MI frequency generally increased as compared with winter time (N<0.01).Such tendency continued with the primary and recurrent MI, as well as, in 1st and 2nd groups. In women younger than 55 years, MI was rarely developed during winter time (N<0.05). In the summer MI complications were observed in 37.3% of cases, where in the winter it was 23.6% (N<0.01). In the winter, despite the peak incidence (65%), the number of complicated MI was almost at the level of the summer period (46.3% and 53.7% respectively), mortality was 30.2% lesser than that of summer period. Indicators of fibrinolysis system and blood rheology in summer period were lowest expressed in elongation clot lysis time as compared with the indicators of cold period (N<0.01), and whole blood viscosity was highest in the summer months with their subsequent reduction by the winter period (N<0.002).

**Conclusion:** Thus, in the summer there is the highest number of deaths among patients with complicated MI, which was accompanied by the lowest values of intravascular platelet aggregation.

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**AAS 18: THE RELATIONSHIP OF FIBROBLAST GROWTH FACTOR 21 WITH CARDIOVASCULAR OUTCOME EVENTS IN THE FENOFIBRATE INTERVENTION AND EVENT LOWERING IN DIABETES STUDY**


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**Background and Aims:** Circulating fibroblast growth factor 21 (FGF21) levels are often elevated in obesity, dyslipidaemia, insulin resistance and type 2 diabetes. This study investigated the relationship of plasma FGF21 levels with cardiovascular events in patients with type 2 diabetes.

**Methods:** Plasma FGF21 levels were measured at baseline in 9,697 study participants with type 2 diabetes from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study by enzyme-linked immunosorbent assay. We assessed the association of FGF21 levels with incidence of different cardiovascular outcomes over 5-years. The primary outcome is total cardiovascular disease (CVD) events, and the secondary outcomes are the four individual components: coronary heart disease (CHD) events, total stroke, CVD mortality, coronary and carotid revascularization. Tertiary outcome is hospitalisation for angina pectoris.

**Results:** Higher baseline FGF21 levels were associated with higher risks of all cardiovascular outcome events after adjusting for the study treatment allocation (all p<0.01). The associations remained significant for total CVD events, and coronary and carotid revascularisation after further adjusting for confounding factors with HR (95% CI) being 1.28 (1.10, 1.50) and 1.26 (1.01, 1.56) respectively, for the highest tertile compared to the lowest tertile (overall effect p=0.002 and 0.007 respectively). The addition of FGF21 levels in a model of total CVD events with established cardiovascular risk factors slightly increased the C-statistic, but resulted in significant integrated discrimination improvement and net reclassification improvement.

**Conclusions:** Higher baseline plasma FGF21 levels were associated with higher risk of cardiovascular events in patients with type 2 diabetes.

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**AAS 19: HEPARANASE IN CELLS OF THE VASCULATURE AND ATHEROSCLEROSIS**

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The endoglycosidase heparanase is involved in a number of physiological processes including wound healing and reproduction, however it has also been associated with the metastatic and angiogenic potentials of tumour cells and has been implicated in several inflammatory diseases. Through cleavage of heparan sulphate, heparanase contributes to the degradation of the extracellular matrix along with the release of sequestered growth factors and cytokines. Although heparanase expression and regulation has been well described in the context of cancer metastasis, little is known about its contribution to the development and progression of atherosclerosis. In order to examine the role of heparanase in atherosclerosis, heparanase expression and activity in cells of the vasculature has been investigated along with the extent of disease development in vivo using heparanase deficient mice on an ApoE<sup>-/-</sup> background. Analysis of the expression and activity of heparanase in cells of the vasculature that constitute atherosclerotic lesions including endothelial cells, smooth muscle cells and macrophages indicates the heparanase is expressed and variably regulated by pro-inflammatory and pro-atherogenic stimuli. Findings from high fat diet experiments using ApoE<sup>-/-</sup> x HPSE<sup>-/-</sup> mice suggest that atherosclerotic lesion development is decreased in the absence of heparanase. Together, these data suggest that heparanase plays an important role in the inflammatory process underlying the development and progression of atherosclerosis, which is the leading cause of death and disability in the western world.
Conclusion: significantly modulated cell-surface expression of LDLR in receptor-negative HoFH fibroblasts. Versus serum + 40 μg/L mevastatin. Alirocumab dose-dependently reversed this effect. Neither recombinant PCSK9 nor alirocumab dose-dependently reduced LDLR cell surface expression in non-FH, HeFH and receptor-defective HoFH fibroblast (p<0.05)

Results: Cell-surface LDLR expression was assessed by flow cytometry. The cells were then incubated with recombinant PCSK9 with or without increasing concentrations of alirocumab. Receiver-negative HoFH patients were grown in 0.5% serum supplemented with increasing doses of mevastatin to maximally up-regulate the LDLR. The cells were then incubated with recombinant PCSK9 with or without increasing concentrations of alirocumab. Cell-surface LDLR expression was assessed by flow cytometry.

Results: LDLR median fluorescence intensity (MFI) in cells treated with 0.5% serum + 40 μg/L mevastatin was 5636±605 in non-FH, 2297±198 in HeFH, 799±89 in receptor-defective HoFH and 216±20 in receptor-negative HoFH subjects. The addition of recombinant PCSK9 dose-dependently reduced LDLR cell surface expression in non-FH, HeFH and receptor-defective HoFH fibroblast (p<0.05 versus serum + 40 μg/L mevastatin). Alirocumab dose-dependently reversed this effect. Neither recombinant PCSK9 nor alirocumab significantly modulated cell-surface expression of LDLR in receptor-negative HoFH fibroblasts.

Conclusion: Greater consumption of reduced fat dairy and vegetables is associated with less arterial stiffening in a cohort of people with diabetes.

AAS 21: EXPRESSION OF LDLR AND INHIBITION OF PCSK9 WITH ALIROCUMAB IN HETEROZYGOUS AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIC PATIENTS

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Background: Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibition with monoclonal antibody inhibitors is a promising therapy for lowering plasma low-density lipoprotein (LDL) cholesterol in familial hypercholesterolemia (FH) patients

Aim: To evaluate whether PCSK9 inhibition with the monoclonal antibody (mAb) alirocumab, is of therapeutic value in heterozygous (HeFH) and homozygous FH (HoFH) patients and whether it is dependent on the functional status of their LDL receptor (LDLR).

Methods: Human dermal fibroblasts (HDF) isolated from control patients, five HeFH patients, six receptor-defective and four receptor-negative HoFH patients were grown in 0.5% serum supplemented with increasing doses of mevastatin to maximally up-regulate the LDLR. The cells were then incubated with recombinant PCSK9 with or without increasing concentrations of alirocumab. Cell-surface LDLR expression was assessed by flow cytometry.

Results: LDLR median fluorescence intensity (MFI) in cells treated with 0.5% serum + 40 μg/L mevastatin was 5636±605 in non-FH, 2297±198 in HeFH, 799±89 in receptor-defective HoFH and 216±20 in receptor-negative HoFH subjects. The addition of recombinant PCSK9 dose-dependently reduced LDLR cell surface expression in non-FH, HeFH and receptor-defective HoFH patients. Alirocumab dose-dependently reversed this effect. Neither recombinant PCSK9 nor alirocumab significantly modulated cell-surface expression of LDLR in receptor-negative HoFH fibroblasts.

Conclusion: PCSK9 inhibition with alirocumab has clinical potential to lower LDL-C in HeFH and receptor-defective HoFH patients.

AAS 22: STATUS OF SERUM PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9 (PCSK9) CONCENTRATION AMONG PATIENTS WITH FAMILIAL HYPERCHOLESTEROLAEMIA PATIENTS WITH AND RELATED UNAFFECTED FAMILY MEMBERS

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Background and Aim: Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is an enzyme that plays a role in lipoprotein metabolism by way of increased degradation of low density lipoprotein receptor (LDLR) that leads to decreased LDL-c clearance. Recent studies suggest the involvement of PCSK9 gene mutations in familial hypercholesterolaemia (FH) that is associated with increased risk of atherosclerosis. However, it is unclear whether related unaffected controls (RUC) carry the same risk. Thus, this study aims to compare serum PCSK9 concentrations between FH, RUC and normal controls (NC).

Methods: 114 FH subjects diagnosed by Simon Broome’s Criteria and 186 age, race, gender-matched NC were recruited. 54 RUC subjects who were the unaffected 1st or 2nd degree relatives of FH patients were also included. Fasting serum samples were collected and analyzed for PCSK9 by enzyme-linked immunosorbent assay method (ELISA).

Results: Serum concentration of PCSK9 was higher in FH compared to NC and RUC (mean ± SEM: 273.9 ng/ml ±
Background and aims: We have previously observed that circulating levels of plasmalogens (phospholipids with anti-oxidant properties) were negatively associated with acute coronary syndrome and unstable angina, suggesting a higher level of oxidative stress in these patients. The modulation of plasmalogen concentration by oral administration of alkylglycerols (precursor to plasmalogen synthesis) has been demonstrated, but its effect on atherosclerosis has not been previously investigated. We hypothesised that increasing the concentration of plasmalogen will attenuate atherosclerosis progression. We aimed to assess the effect of plasmalogen enrichment on atherosclerosis progression in murine models of atherosclerosis with differing levels of oxidative stress.

Methods and results: Six-week old ApoE- and ApoE/GPx1-deficient mice were fed a high-fat diet (HFD) with or without 2% batyl alcohol (BA, 18:0 alkylglycerol) for 12 weeks. Analysis of lipids in plasma and heart via liquid chromatography electrospray ionisation mass spectrometry showed that supplementation of BA to ApoE- and ApoE/GPx1-deficient mice resulted in increases in the total plasmalogen concentration in both tissues (P<0.001 for both genotypes). En face analysis showed that the mice fed HFD without BA developed extensive atherosclerotic plaques throughout the aorta. This was reduced by 70% (P<0.001) in the BA-treated mice for both genotypes. A reduction in plaque was also seen in the aortic sinus of the treated ApoE-deficient mice (-40%, P<0.01) however, the reduction in the treated ApoE-deficient mice was not significant (-12%, P=0.18). Aorta and aortic sinus were immunostained for the oxidative stress marker, nitrotyrosine, and the inflammatory marker, VCAM-1. Compared with the corresponding untreated groups, the BA-treated ApoE/GPx1-deficient mice showed a greater decrease in nitrotyrosine formation in the aorta (-78%, P<0.001) and VCAM-1 expression in the aortic sinus (-28%, P<0.05) than treated ApoE-deficient mice (-2%, P=0.90 for nitrotyrosine formation, and -15%, P=0.40 for VCAM-1 expression).

Conclusions: Plasmalogen enrichment via BA supplementation attenuated atherosclerosis in ApoE- and ApoE/GPx1-deficient mice, with a greater effect in the aortic sinus of the latter group. Supplementation with BA may exert its greatest protective effect in environments of elevated oxidative stress and inflammation as seen in the ApoE/GPx1-deficient mice. Plasmalogen enrichment may represent a viable therapeutic strategy to prevent atherosclerosis and reduce cardiovascular disease risk.
AAS 25: LOW DOSE NITRATE IMPROVES ENDOTHELIAL FUNCTION IN THE APOE-/- MOUSE

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Background and Aims: Nitric oxide (NO) is an important vascular signaling molecule that plays a role in the control of vascular function and the prevention of cardiovascular disease. NO can be synthesized endogenously by endothelial nitric oxide synthase (eNOS), and this accounts for up to 70% of the body's NO requirements. An alternate pathway is exogenous dietary nitrate, which can be converted to nitrite, and then stored or used immediately. This pathway accounts for up to 30% of the body's NO supply. Atherosclerosis is associated with endothelial dysfunction and subsequent lesion formation. This is thought to arise due to a reduction in the bioavailability and/or bioactivity of endogenous NO. Therefore, the aim of this study was to determine if dietary nitrate could protect against endothelial dysfunction and lesion formation in the ApoE-/- mouse fed a high fat diet (HFD).

Methods: ApoE-/- were randomized to receive either (i) high nitrate (10mmol/kg/day, n=12), (ii) medium nitrate (1mmol/kg/day, n=8), or (iii) low nitrate (0.1mmol/kg/day, n=8) supplemented drinking water for 10 weeks. A group receiving sodium chloride supplemented drinking water (n=10) served as control, while a group of C57BL6 mice (n=6) received regular water and served as a healthy reference group. All mice were fed a HFD and at the end of the 10 week period, had their ex vivo endothelial function assessed using isolated aortic rings.

Results: Acetylcholine (Ach) mediated vessel relaxation was significantly impaired in ApoE-/- mice versus C57BL6. Mice supplemented with low or medium dose nitrate showed significant improvements in Ach-mediated vessel relaxation compared to ApoE-/- mice given the high nitrate dose or ApoE-/- mice given sodium chloride. Plasma nitrate and nitrite levels were significantly increased in all three groups fed the nitrate-supplemented water. There appeared to be no significant effect of high dose nitrate supplementation on aortic lesion formation or F2-isoprostanes, with results for medium and low dose nitrate, still pending.

Conclusions: Low and medium dose nitrate, but not high dose nitrate, appears to have beneficial effects on Ach-mediated vessel relaxation. This may be due to down-regulation of eNOS by high dietary nitrate. These results have significant implications for nitrate supplementation in humans.

AAS 26: MONOCYTES SHOW INCREASED M1 AND DECREASED M2 MARKERS WITH ATHEROSCLEROSIS

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Background and aims: Macrophages play a major role in atherosclerotic plaque stability with M1 macrophages thought to promote instability and M2 macrophages stability. Their precursors, the monocytes, are heterogenous consisting of classical (~85%), intermediate and nonclassical monocytes. Expansion of the latter two has been reported in CVD and these are therefore implicated in atherosclerosis. However, changes in monocyte profile, rather than counts, are rarely examined in individual subsets. As such, we sought to determine whether, and how, specific monocyte subsets differ in inflammatory profile with atherosclerotic risk, and between patients and controls.

Methods: Blood was collected from patients and controls and lipid analysis was performed. Monocyte subset expression of M1 and M2 markers was determined by whole blood flow cytometry. Luminex assays were used to determine serum M1 chemokine concentrations.

Results: Consistent with the literature, an increase in the intermediate subset was observed in patients. This subset appeared to be more M1-like as evident by increased expression of the M1 marker CD282 a decreased expression of M2 markers (CD93, CD163) and a higher CD86/CD163 ratio (all p<0.05). This could suggest an expansion in the intermediate subset in patients results in an increased number of circulating M1-like cells. Interestingly, though the intermediate subset appears more inflammatory, the classical subset became more M1-like with atherosclerotic risk. This was seen by the negative correlation between CD86/CD163 and HDL and, conversely, the positive correlation between CD163 and HDL, with CD93 showing a similar trend. This suggests that, with atherosclerotic risk, M1 markers increase and M2 markers decrease. In patients, a further skewing of the classical subset towards an M1-like phenotype was seen by a significantly higher CD86/CD163 ratio compared to controls. Luminex assays revealed that patients had significantly higher M1 chemokines than controls with a 1.5 fold higher Mip3b (p<0.05) and a 2-fold higher MIG (p<0.01). This indicates a more M1-like environment in the patients which may result from, or lead to, increased presence of M1-like cells.

Conclusions: The classical monocyte, despite not showing a proportional expansion in atherosclerosis, shows a shift towards a more M1-like phenotype with increased atherosclerotic risk and presence of disease. As this is the major monocyte subset, the M1 shift may have an additive, or greater, impact on atherosclerosis than increases in intermediate or nonclassical cell number.
AAS 27: GLYCINE AMIDINOTRANSFERASE VARIANT RS9806699 IS ASSOCIATED WITH STATIN-INDUCED MYALGIA

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Background: Statins are widely prescribed to lower low-density lipoprotein cholesterol and cardiovascular disease risk. Although serious rhabdomyolysis is very rare with statins, reports have shown that up to 10 – 15% of exposed patients develop myalgia, which often necessitates dose reductions or cessation of treatment. Genetic variability may be an important risk factor for statin-related muscle side effects. Gene expression studies in lymphoblastoid cells lines derived from simvastatin-treated patients, identified an expression quantitative trace locus that interacted with statin exposure; the SNP rs9806699 for the gene that encodes glycine amidinotransferase (GATM), a rate-limiting enzyme in creatine synthesis. This locus was found to be associated with a reduced incidence of statin-induced muscle toxicity in patients with elevated creatine kinase levels, but was not replicated in patients with rhabdomyolysis. The relevance of the GATM gene variant rs9806699 in statin-induced myalgia patients without creatine kinase elevations has yet to be elucidated.

Aims: We studied 186 patients from a single lipid clinic at Christchurch Hospital. Sixty-seven statin-intolerant patients, defined as having symptomatic muscle weakness, tenderness and/or pain on statin therapy and again on re-challenge, or using two different statins, and 57 statin tolerant controls from the lipid clinic, who were taking at least 80mg of simvastatin or atorvastatin for ≥3 months with no reported myalgia symptoms and 62 consecutive outpatient controls.

Methods: DNA from these cases and controls was genotyped at the GATM locus using ABI sequencing of PCR products. Associations were evaluated using chi-square analysis or Fisher’s exact test as appropriate, and presented as odds ratios (OR) and 95% Confidence Intervals (CI).

Results: The minor allele frequencies for GATM SNP rs9806699 was 0.20, 0.36 and 0.32 for statin intolerant cases, statin tolerant controls and consecutive outpatient controls, respectively. The minor allele for this gene variant was associated with a reduced incidence of statin-induced myalgia compared to statin tolerant controls (OR=0.42, 95% CI=0.24 – 0.74, P=0.0029). Furthermore, the minor allele for the SNP rs9806699 at GATM locus was also associated with a reduced odds of statin-induced myalgia compared to statin consecutive outpatient controls (OR=0.53, 95% CI=0.30 – 0.93, P=0.031).

Conclusions: The presence of the minor allele for the GATM SNP rs9806699 in the muscle may influence the production of creatine products, and protect from statin induced-myalgia.
AVBS 01: TRAIL PROMOTES ANGIOGENESIS AND ISCHEMIA-INDUCED NEOVASCULARISATION VIA NOX4, H2O2 AND NITRIC OXIDE-DEPENDENT MECHANISMS

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Background and Aims: Angiogenesis and neovascularization are essential processes in ischemia-related conditions, such as cardiovascular disease and diabetes. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) not only induces endothelial cell (EC) death and inhibits angiogenesis, but also promotes EC migration, invasion and proliferation in vitro. These seemingly opposite effects make its role in angiogenesis in vivo unclear. Using TRAIL-/- and wild-type mice, we sought to determine the role of TRAIL in angiogenesis and neovascularisation in vivo and also establish mechanisms in vitro.

Methods and Results: Hindlimb ischemia in TRAIL-/- mice showed reduced vascularisation assessed by real-time in vivo 3D Vevo ultrasound imaging, and CD31 staining 28 d after ischemic injury. Reduced capillary formation and increased apoptosis was also evident 3 d after ischemic surgery in TRAIL-/- muscles. Fibroblast growth factor-2 (FGF-2) is a potent angiogenic factor that regulates TRAIL expression in vascular smooth muscle cells. Not only was FGF-2 mRNA reduced in 3 d ischemic hindlimbs of TRAIL-/- mice, FGF-2 increased TRAIL gene expression in ECs. Consistent with these findings, FGF-2-inducible proliferation, migration and tubule formation was inhibited with siRNA targeting TRAIL. NADPH oxidase 4 (NOX4) was recently implicated to play a protective role in cardiovascular disease. Indeed both FGF-2 and TRAIL significantly increased NOX4 mRNA and protein expression. FGF-2 and TRAIL-inducible proliferation, migration and tubule formation was also inhibited with siRNA targeting NOX4. Furthermore, FGF-2 and TRAIL’s pro-angiogenic activity in vitro was not only blocked with PEG-catalase, a H2O2 scavenger, but also blocked with L-NAME, an inducible proliferation, migration and tubule formation was also inhibited with siRNA targeting TRAIL. NADPH oxidase 4 (NOX4) was recently implicated to play a protective role in cardiovascular disease. Indeed both FGF-2 and TRAIL significantly increased NOX4 mRNA and protein expression. FGF-2 and TRAIL-inducible proliferation, migration and tubule formation was also inhibited with siRNA targeting NOX4. Furthermore, FGF-2 and TRAIL’s pro-angiogenic activity in vitro was not only blocked with PEG-catalase, a H2O2 scavenger, but also blocked with L-NAME, a nitric oxide synthase inhibitor. In support, TRAIL promoted nitric oxide release from ECs.

Conclusion: This is the first demonstration showing that TRAIL promotes angiogenesis in vivo. We show for the first time that the TRAIL stimulates NOX4 expression to mediate nitric oxide-dependent angiogenic effects. This has significant therapeutic implications such that TRAIL may improve the angiogenic response to ischemia and increase perfusion recovery in patients with CVD and diabetes.

AVBS 02: TRAIL-DEFICIENCY CAUSES INSULIN RESISTANCE, TYPE-2 DIABETES, AND INFLAMMATION IN THE VESSEL WALL

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Background and Aims: We have previously shown that TNF-related apoptosis-inducing ligand (TRAIL) is protective in both cardiovascular disease and diabetes. Insulin resistance is a characteristic of type-2 diabetes, and whether TRAIL regulates insulin resistance and inflammation in the vessel wall is unknown.

Methods: 6 week old mice were placed on a high fat diet (HFD) for 12 weeks. Plasma chemistries were assessed; glucose and insulin tolerance tests performed. Histological GLUT4 expression in skeletal muscle was assessed. Expression of hepatic genes involved in lipogenesis and glucose metabolism were measured by qPCR. Aortic inflammatory marker expression was also measured.

Results: In response to a HFD, WT mice had increased aortic IL-1β, MCP-1, IL-6, TNF-α and TRAIL mRNA. In contrast, circulating TRAIL levels were reduced. Compared to HFD-fed WT, TRAIL-/- mice had significantly increased plasma glucose, insulin, and cholesterol. Insulin intolerance was exacerbated with TRAIL-deficiency, with reduced GLUT4 expression and glucose uptake in skeletal muscle. TRAIL-/- liver showed increased SREBP1 and HMGCoAR mRNA, with PEPCK induced by insulin. In response to insulin, TRAIL-/- aortas displayed impaired vasorelaxation and reduced aortic p-Akt expression, associated with > 20-fold increased aortic IL-1β, IL-6, and TNF-α expression. Interestingly, acute insulin increased TRAIL mRNA expression and proliferation of vascular smooth muscle cells, whilst chronic exposure down-regulated TRAIL and promoted apoptosis; a finding dependent on the transcription factor Sp1.

Conclusion: We show that TRAIL protects against insulin resistance, type-2 diabetes and vascular inflammation in response to a HFD, and that insulin positively and negatively regulates TRAIL expression to modulate VSMC proliferation and/or apoptosis. This mechanism may offer novel therapeutic solutions to combat diabetic vascular diseases.

AVBS 03: THE PROTECTIVE PROPERTIES OF TRAIL IN ATHEROSCLEROSIS AND DIABETES FROM DIFFERENT SOURCES: A BONE MARROW TRANSPLANT MODEL

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Background and Aims: We have previously shown that TNF-related apoptosis-inducing ligand (TRAIL) is protective in both atherosclerosis and diabetes in mice. However, to use TRAIL as a potential therapy in the treatment of these diseases, it is necessary to determine if different sources offer different protective features. The aim of this study was to determine whether the cellular source of TRAIL affects its protective function, using a bone marrow transplant model.
Methods and Results: 8 week old TRAIL-/ApoE-/ mice, following a lethal dose of radiation, were transplanted with bone marrow (BM) from either TRAIL-/ApoE-/ donors, maintaining whole body TRAIL deficiency (TRAIL-/BM-); or ApoE-/ donors, restoring TRAIL expression in cells originating in the bone marrow (TRAIL-/BM+/+). ApoE-/ mice were also irradiated and given a BM transplant from TRAIL-/ApoE-/ donors; leading to TRAIL deficiency in cells originating in the bone marrow, whilst TRAIL expression in tissues remained unaltered (TRAIL+/BM-/). Mice were allowed to recover for 4 weeks to establish chimerism. They were then placed on a high fat “Western style” diet (HFD) for 12 weeks. No differences in weight gain, plasma glucose, insulin or cholesterol levels were observed between groups. Compared to TRAIL-/BM-/ mice, which displayed larger acellular plaques, TRAIL-/BM+/+ mice had significantly reduced atherosclerotic lesions in brachiocephalic arteries, suggesting that BM-derived TRAIL is protective of atherosclerosis. Interestingly, aortic mRNA expression of CD68, which binds LDL and is frequently used as a marker of macrophages, and CD11c, considered a marker of dendritic cells, were significantly elevated in TRAIL-/BM+/+ mice. Further, these animals had significantly increased aortic PPARγ mRNA expression. Of note, TRAIL+/BM-/ mice displayed a slight reduction in plaque size, suggesting that tissue TRAIL expression may also protect against atherogenesis. More importantly, these mice showed significant improvement in their ability to clear glucose following a glucose tolerance test, implying that tissue TRAIL is important in glucose homeostasis.

Conclusions: Here we show that different sources of TRAIL protect against atherosclerosis and diabetes. These findings need to be taken into account when considering TRAIL as a potential therapeutic option for these disease states. The contribution of TRAIL from the BM vs. the tissue, in disease, requires further study.

AVBS 04: USING PIOGLITAZONE TO IDENTIFY NEW TARGETS FOR ANEURYSM TREATMENT – THE ROLE OF EGR1 IN AN EXPERIMENTAL MURINE MODEL OF AORTIC ANEURYSM


Background and Aims: Peroxisome proliferator-activated receptor γ (PPARγ) agonists inhibit Angiotensin II-induced experimental abdominal aortic aneurysms. Since macrophage infiltration to the vascular wall is an early event of the pathology, we set out to explore the effects of the PPARγ agonist, pioglitazone, on Angiotensin II-treated monocytes, in order to identify new targets for aneurysm therapy.

Methods and Results: Using microarray-based expression profiling, we found that a number of aneurysm-related gene changes affected by Angiotensin II were modulated following addition of pioglitazone. Among those, polycystic kidney disease-1 (PKD1) was significantly up-regulated (P<0.05). Analysis of the PKD1 proximal promoter revealed a putative Early Growth Response-1 (EGR1) binding site, which was confirmed by chromatin immunoprecipitation (ChIP) and quantitative PCR. Analysis of publicly available ChIP-sequencing data revealed that this putative binding site overlapped with a conserved EGR1-binding peak present in five other cell lines. Quantitative real time PCR showed that EGR1 suppressed PKD1, while Angiotensin II significantly up-regulated PKD1, an effect counteracted by pioglitazone. Conversely, in EGR1 short-hairpin RNA lentivirally-transduced THP-1 cells, reduced EGR1 led to significant up-regulation of PKD1, especially after treatment with pioglitazone. Additionally, in chimeric mice, where EGR1-deficient bone marrow cells had been used for re-population of the wild-type haemopoietic compartment after irradiation, the incidence of CaCl₂-induced aneurysms was significantly reduced.

Conclusions: We show that PKD1 is an EGR1-target gene, with EGR1 suppressing its expression in differentiating monocytes. Angiotensin II treatment up-regulates the expression of PKD1 and pioglitazone mediates the opposite effect. EGR-1 deficiency in the haematopoietic compartment abolishes experimental aneurysm formation.

AVBS 05: VEGF AND VEGF RECEPTOR IN OVARIAN HYPERSTIMULATED PREGNANCY

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Vascular endothelial growth factor (VEGF) is an important protein both in the ovary, during follicular maturation, and in the uterus during pregnancy, for blastocyst implantation and placental development. During fertility treatments, such as in vitro fertilisation (IVF), a technique known as ovarian hyperstimulation (OH) is used to stimulate the growth of several follicles within the ovary resulting in superovulation. However, this treatment alters the normal hormonal profile, resulting in changes in the expression of several proteins within the uterus, including VEGF. These changes consequently result in reduced endometrial receptivity to blastocyst implantation and may contribute to the higher incidence of placental anomalies in IVF patients.

A recently developed rat OH model is a useful technique in studying the changes in the endometrium and ovaries in response to fertility drugs, similar to those used in human IVF procedures, however studies of VEGF in this model are limited. Female rats with at least 2 continuous 4-day oestrous cycles were IP injected with 20 IU of equine serum gonadotropin followed by 20 IU of human chorionic gonadotropin 24-hours later. Non-pregnant rats were sacrificed that evening during proestrus, whereas rats designated for pregnancy were mated overnight and sacrificed on days 1, 6 and 9 of OH and normal pregnancy. The uterine and ovarian tissues were then collected and processed for immunohistochemistry and western blot analysis.

So far immunostaining for VEGF suggests the localisation is altered in uterine epithelial cells of OH pregnancy compared to normal pregnancy from as early as day 1, the time of fertilisation. Western blot analysis also shows that the amount of VEGF is reduced in OH uterine epithelial cells at this time. Changes are also seen in both immature and ruptured follicles in the ovaries at the time of fertilisation, and in the corpus luteum at day 6, the time of implantation.
Western blot analysis shows a similar reduction of VEGFR2 in uterine epithelial cells of OH pregnancy at the time of implantation, when compared to normal pregnancy. Thus the lower levels of VEGF and VEGFR2 seen in OH compared to normal pregnancy may influence uterine receptivity and early blood vessel remodelling in the placenta. The changes in the ovaries may also influence vascular remodelling around the follicle, potentially effecting oocyte maturation. Therefore, these changes may contribute to lower uterine receptivity and oocyte quality following hormonal stimulation in IVF patients.

AVBS 06: SPHINGOSINE KINASE-2 CONTROLS VASCULAR ENDOTHELIAL CELL BARRIER INTEGRITY

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Endothelial cells play a fundamental role in regulating the vascular barrier, the integrity of which is essential for co-ordinating the movement of blood components and leukocytes into extracellular tissues. A key mediator of vascular barrier integrity is the bioactive lipid sphingosine-1-phosphate (S1P), which signals through its G-protein coupled receptors found on the endothelial cell surface. The lipid kinases sphingosine kinase-1 and sphingosine kinase-2 (SK-1 and SK-2) are involved in the conversion of sphingosine to S1P and previous work has implicated SK-1, but not SK-2, in the signalling pathways that regulate barrier integrity. The aim of this study was to determine the role of SK-2 in regulating vascular barrier integrity. An in vitro model was utilised, employing primary human umbilical vein endothelial cells (HUVEC) and a cell impedance assay based on the xCELLigence technology platform. Inhibitors to SK-1 (SKI and PFS43) and SK-2 (ABC294640) were used to determine and compare the roles of SK-1 and SK-2 on endothelial cell barrier integrity. The SK-1 inhibitor SKi failed to induce any changes in barrier integrity, whilst PF543 only induced a modest decrease in barrier integrity. In contrast, treatment with the SK-2 inhibitor ABC294640 resulted in a significant decrease in barrier integrity. This data was further validated using intravital microscopy on Sphk2 knock-out mice. This data suggests for the first time that SK-2, along with SK-1, plays a significant role in regulating endothelial cell barrier integrity. This work may help in the identification of treatments for conditions such as allergy, including anaphylaxis, where changes in vascular barrier function can have detrimental health effects.

AVBS 08 PROMOTION OF NITROSO-REDOX BALANCE BY BETΑ 3 ADRENOCEPTOR AGONISM: THERAPEUTIC IMPLICATIONS FOR CARdiovascular COMPLICATIONS OF DIABETES IN HUMANS

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Rationale: Disrupted balance between NO and O₂⁻ is central in pathobiology of diabetes-induced vascular dysfunction. We examined if stimulation of β3 adrenergic receptors (β3 ARs), coupled to endothelial nitric oxide synthase (eNOS) activation, would re-establish NO/O₂⁻ balance, relieve oxidative inhibition of key caveolar proteins and protect against diabetes-induced cardiovascular dysfunction.

Methods/Results: A hyperglycemic, hyperinsulinemic state was established in male White New Zealand rabbits by infusion of the insulin receptor antagonist S961 (12 μg/kg/h). Diabetes induced NADPH oxidase-dependent glutathionylation (GSS-) of the caveolar proteins Na⁺+K⁺ pump's β1 subunit and eNOS in aorta, an oxidative modification that inhibits the pump and uncouples eNOS. Consistent with this, diabetes was associated impaired endothelium-dependent vasorelaxation. Selective β3 AR agonist CL316243 (CL, 40 μg/kg/h) restored NO levels analysed by spin-trapping of NO-Fe(DETC)2 complexes; decreased diabetes-induced elevation in O₂⁻ measured by HPLC analysis of dihydroethidium oxidation products, improved endothelium-dependent vasorelaxation, and restored the Na⁺+K⁺ pump function. These effects were mediated by CL abolishing diabetes-induced increase in eNOS-GSS and β1-GSS by suppressing diabetes-induced NADPH oxide activation and further amplified by promotion of de-glutathionylation via enhancement in association of glutaredoxine-1, the enzyme catalysing de-glutathionylation, with eNOS and Na⁺+K⁺ pump. eNOS-GSS was higher in vessels of diabetic patients undergoing CABG, which was reduced with CL exposure, indicating the direct relevance of findings to human diabetes.

Conclusion: β3 AR activation re-established nitroso-redox balance and relieved oxidative inhibition of key caveolar proteins in diabetes. Our findings in human vasculature suggest β3 AR agonists are promising in treatment of diabetes-induced vascular complications.

AVBS 09: MONOCYTE CHEMOATTRACTANT PROTEINS DIFFERENTIALLY AFFECT MACROPHAGE POLARISATION

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Background and Aims: Advanced atherosclerotic plaques are associated with an increased ratio of pro-
inflammatory M1 to anti-inflammatory M2 macrophages and increased activity of the reactive oxygen species generating enzyme, NOX2 NADPH oxidase. The CCR2 chemokines, monocyte chemoattractant proteins (MCPs) 1, 2 and 3, promote monocyte infiltration into the vascular wall, thus playing a crucial role in atherogenesis. However, little is known of their effect on macrophage polarisation. Therefore we aimed to elucidate the effects of MCP-1, 2 and 3 on macrophage polarisation by measuring expression of M1 and M2 markers and superoxide generation.

**Methods:** Phorbol-12,13-dibutryrate (PDBu, 10nM, 24 hrs)-differentiated human monocytes (THP-1) were left untreated (M0), treated with 10ng/ml lipopolysaccharide and 5ng/ml interferon-gamma (LPS-IFN-γ, 48 hrs) or 25ng/ml interleukin-4 (IL-4, 48hrs) for M1 and M2 positive controls respectively, or treated with MCP-1, MCP-2, or MCP-3 (100nM, 48 hrs). Quantitative real time PCR confirmed macrophage polarisation in terms of M1 (TNFα, IL-1β) and M2 (MRC1) marker, and NADPH oxidase isoform and subunit (NOX2, p47phox) mRNA expression. PDBu-stimulated superoxide production was detected by L012-enhanced chemiluminescence.

**Results:** The mRNA expression of M1 markers TNFα and IL-1β were increased in the M1 positive control (8-fold, P<0.01; n=7) and following treatment with MCP-1 (5-fold, P<0.01; n=7) and MCP-2 (4-fold, P<0.05; n=6) but not MCP-3. The M2 marker MRC1 was increased in the M2 positive control (5-fold, P<0.01, n=6) but not by treatment with any MCP. In addition, M1 macrophages had increased expression of the NOX2 (2-fold, P<0.01; n=6) and p47phox (7-fold, P<0.01; n=6) subunits of the NOX2 oxidase complex.

**Conclusions:** Our findings suggest that MCPs can differentially affect macrophage polarisation. Both MCP-1 and MCP-2, but not MCP-3, promote a M1-like pro-inflammatory macrophage phenotype. Thus targeting MCP-1 and MCP-2 may have therapeutic potential to stabilize atherosclerotic plaques by reducing the population of M1 macrophages.

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**AVBS 10: TETRASPANIN TSSC6 AND P2Y12 RECEPTOR PARTICIPATE IN A COMMON PATHWAY TO REGULATE THROMBUS GROWTH AND STABILITY**

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**Background:** Tumor-suppressing subchromosomal transferable fragment cDNA 6 (TSSC6) expressions are restricted to hematopoietic organs and tissues where it may play a role in hematopoietic-cell function. The ADP purinergic receptor P2Y12 is mainly expressed by platelets with important clinical significance as a target for several clinically approved antithrombotic agents.

**Aim:** To investigate the functional importance between TSSC6 and P2Y12 receptor in platelet function, using wild-type or TSSC6-deficient mice treated with either PBS or 50 mg/kg clopidogrel.

**Methods:** The platelet granule release was assessed by measuring the α and dense granule release. The postoccupancy events of integrin αIIbβ3 were determined using clot retraction, platelet aggregation and platelet spreading on fibrinogen. The “inside-out” integrin αIIbβ3 signalling was examined using FITC-fibrinogen and JON/A mAb expression

**Results:** TSSC6-deficient mice treated with clopidogrel exhibited further impaired in kinetics of clot retraction, platelet aggregation at different doses of collagen and platelet spreading on fibrinogen compared to those with solitary TSSC6 knockout or P2Y12 receptor blockade. Neither alpha or dense granule secretion nor “inside-out” integrin αIIbβ3 signalling were affected

**Conclusion:** These preliminary data demonstrated a functional relationship between TSSC6 and P2Y12 receptor in platelets in regulating thrombus growth.

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**AVBS 11: EXPLORING THE EFFECT OF COLCHICINE ON PLATELET FUNCTION**

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**Background and Aims:** Colchicine is commonly used in the treatment of gouty arthritis and familial Mediterranean fever. More recently it has been used for secondary prevention of coronary events. We hypothesised that some of the apparent clinical benefits may be due to inhibition of platelet activation.

**Methods:** Platelet aggregation in response to collagen and adenosine diphosphate (ADP) was examined both in whole blood and platelet rich plasma (PRP). Platelet activation markers were assessed by flow cytometry after stimulation with arachidonic acid (AA), thrombin receptor activating peptide (TRAP) or ADP. Low dose colchicine (20 nM) representing typical plasma concentrations to which the platelet surface is exposed and high dose colchicine (1-2 mM) representing concentrations which are expected to affect microtubule function were both examined. Paired t-test, Friedman ANOVA and Dunn’s multiple comparison tests were used as appropriate.

**Results:** In whole blood, 20 nM colchicine decreased aggregation in response to collagen (70.1±5.5 vs 63.3±5.0, p=0.041, n=10) (mean±SEM), but not to ADP (34.2±4.2 vs 32.3±3.6, p=ns, n=10). In PRP 20 nM colchicine decreased aggregation in response to both collagen (78.6±3.1 vs 73.2±3.7, p=0.002, n=11) and ADP (77.7±4.0 vs 71.9±4.5, p=0.04, n=11). Upregulation of CD62P expression by AA (p=0.0008), TRAP (p=0.009) and ADP (p=0.009), were significantly inhibited by 2 mM colchicine. Upregulation of the platelet expression of MMP regulator CD147 and ADP-induced GPIIb/IIa
conformational change (PAC-1 binding) were inhibited by 2 mM colchicine (p=0.02 and p=0.0008 respectively). Upregulation of CD9 and CD69 expression by TRAP (p=0.009 and p=0.04 respectively) were also inhibited by 2 mM colchicine.

**Conclusions:** Low concentrations of colchicine inhibit collagen- and ADP-induced platelet aggregation, whereas higher concentrations inhibit expression of CD62P, PAC-1 and CD147. Colchicine-mediated inhibition of platelet activation may contribute to the favourable effects of colchicine on vascular events.

**AVBS 12: THE ROLE OF MICROPARTICLES IN GLOBAL FIBRINOLYSIS: DEPLETION AND REPLETION EFFECTS ON THE OVERALL HAEMOSTATIC POTENTIAL ASSAY**

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**Background and Aims:** The overall haemostatic potential (OHP) assay is a global coagulation assay with the ability to detect hypercoagulability and hypofibrinolysis in patients at high risk of thrombosis including those with coronary artery disease. The mechanisms underlying abnormalities in global coagulation and fibrinolysis may include contributions from blood cell-derived microparticles (MP). We therefore aimed to assess the role of MP in the OHP assay.

**Methods:** Using sequential ultracentrifugation, MP and then exosomes were removed from the platelet-depleted plasma of ten healthy donors. The resulting plasma fractions were then assessed for coagulability using the OHP assay, in which tissue factor is used to trigger fibrin generation and tissue factor/tissue plasminogen activator is used to trigger fibrinolysis in a 96-well plate. The timecourse curves generated are described through parameters including the maximum rate of fibrin generation (MaxSlope) and the maximum rate of fibrinolysis (MinSlope). Flow cytometric analysis of the fractions was carried out using fluorescently conjugated antibodies and submicron calibration beads for gating standardisation.

**Results:** MP-depleted plasma demonstrated a significantly depressed rate of fibrin generation (mean±SD, MaxSlope 43±24 vs 93±28 mOD/min, p=0.0009, paired t-test) and significantly decreased rate of fibrinolysis (MinSlope 24±22 vs 52±15 mOD/min, p=0.0022 by paired t-test) compared to whole plasma. Exosome-depleted plasma had no fibrin generation activity over 100 minutes in any donor. However, repletion of the exosome-depleted plasma with the MP-rich fraction caused a significantly higher increase of fibrin generation (to 60±30 % of baseline) than repletion with the exosome-rich fraction (repletion to 18±18 % of baseline, p=0.0008). Preliminary flow cytometry investigation of markers present on the MP in each fraction revealed a significantly higher number of platelet-derived (CD41+-), endothelial-derived (CD31+/CD41-), annexin V-binding and tissue factor positive microparticles in the MP-rich fraction than the other fractions.

**Conclusions:** These preliminary findings indicate a role for platelet-derived microparticles in coagulation and fibrinolysis as assessed by the OHP assay.

**AVBS 13: PREIMPLANTATION FACTOR (PIF) INHIBITS MONOCYTE MIGRATION AND PREVENTS PROGRESSION OF ATHEROSCLEROSIS IN APOLIPOPROTEIN E-DEFICIENT MICE**

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**Background and Aims:** Atherosclerosis is well established as a chronic inflammatory disease, where high circulating lipids and inflammatory mediators are major contributors to plaque formation. The embryo-secreted peptide PreImplantation Factor (PIF) promotes embryo implantation, and modulates the maternal immune response to achieve embryo tolerance without compromising the immune defense against pathogens. These characteristics highlight PIF as a potential immunomodulator against chronic inflammatory diseases. Here we examined the effect of synthetic PIF on progression of atherosclerosis in ApoE-/- mice.

**Methods:** Eight-week old ApoE-/- mice were maintained on a high-fat diet (22% fat, 0.15% cholesterol) and received either PBS control, scrambled PIF control (0.3-3mg/kg) or PIF (0.3-3mg/kg) for 7 weeks. PIF’s effects on lesion size, lesion composition, pro-inflammatory molecule expression and plasma lipid profile were assessed.

**Results:** After 7 weeks of chronic treatment, PIF significantly reduced atherosclerotic lesion burden in the aortic arch and aortic sinus in a dose-dependent manner. Immunohistochemical analysis of atherosclerotic lesions revealed a corresponding decrease in lipid content (oil red O), VCAM-1 and MCP-1 expression, and CD68+ macrophage accumulation; however PIF did not alter plasma lipid levels. For further mechanistic insight, we explored PIF’s effect on monocyte function. PIF inhibited leukocyte extravasation (thioglycollate-induced peritonitis) and LPS-induced rolling and adhesion of monocytes in the mesenteric venules of C57BL/6 mice (intravital microscopy) while in vitro, PIF reduced migration of THP-1 monocytes in a chemotaxis assay.

**Conclusions:** Chronic administration of PIF reduces atherosclerosis lesion burden and inflammation in high-fat fed ApoE-/- mice. We have identified that PIF exerts an inhibitory effect on monocyte migration, thus providing a potential mechanism of action. Overall, our findings identify PIF as a drug candidate against atherosclerosis with the promise of being well tolerated.
The endothelium of intact healthy adult arteries does not express protein for the large conductance calcium-activated potassium channel (BKCa). However, BKCa-alpha and -beta1 protein, as the pore and regulatory subunits, respectively, form functional channels in isolated vascular endothelial cells, and can be upregulated in chronic hypoxia, suggesting a stress-induced phenotype. Using characterized antibodies to BKCa and b1 (Alomone APC-107 to aas 1184-1200; and Merck (Garcia NJ) to aas 118-132, respectively) and confocal-immunohistochemistry, the distribution and expression profile of BKCa and b1 protein was determined in proximal middle cerebral artery (MCA) of 8-10wk male SD rats in endothelin (120 pM bolus)-induced stroke and hypoxia (8%O2/1h/d for 5d; 24h post-stroke). A semi-quantitative association of relative fluorescence intensity (FI) to protein density was determined, with baseline as secondary only. In MCA of untreated and saline-treated control, endothelial FI was the same as secondary alone. In endothelium of MCA from stroke, hypoxia and hypoxia following stroke, endothelial expression was 2157±345; 1878±423; 1765±128 relative FI units, respectively (n=3-4, from different animals) above control. Smooth muscle expression was the same in untreated and saline-treated control and the three treatment groups (P<0.05; albeit at ~2.5-fold higher than in the endothelium). Diffuse endothelial BKCa and b1 were evenly distributed at the endothelial membrane, with higher level FI near cell borders, at untreated and saline-treated control and the three treatment groups (P<0.05; albeit at ~2.5-fold higher than in the endothelium). Diffuse endothelial BKCa and b1 were evenly distributed at the endothelial membrane, with higher level FI near cell borders, at untreated and saline-treated control and the three treatment groups (P<0.05; albeit at ~2.5-fold higher than in the endothelium).

AVBS 14: ACUTE HYPOXIA AND FOCAL CEREBRAL ISCHEMIA RESULT IN ENDOTHELIAL BKCA-ALPHA AND -BETA1 PROTEIN EXPRESSION IN INTACT ADULT RAT MIDDLE CEREBRAL ARTERY ENDOTHELIUM

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Abdominal aortic aneurysm (AAA) is an inflammatory disease leading to increased risk of aortic rupture. AAA is often asymptomatic and identified during imaging for other purposes. Drug therapies for AAA often lack efficacy, and so new treatment strategies given at an early stage of disease are needed. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) and their metabolite resolvin D1 have anti-inflammatory and pro-resolving activities. This study tested the hypothesis that dietary supplementation of apolipoprotein E deficient (ApoE-/-) mice with n-3 PUFAs may protect against an early-phase inflammatory response induced by 2-day infusion of mice with angiotensin II (AngII; a model of pre-AAA).

ApoE-/- and C57bl/6 mice (male, 3-4 weeks) were fed a low (LFA; 0.14%) or high n-3 PUFA diet (HFA; 0.70%) for 8 weeks, and then infused with AngII (1000 ng/kg/min; ApoE-/- mice) or saline (C57bl/6 mice), for 2 days. Aortas were examined for dissection, inflammatory cell infiltration, and levels of inflammatory cell superoxide, NADPH oxidase (Nox2), and matrix metalloproteinase MMP-9. Plasma resolvin D1 levels were determined using ELISA.

4/10 ApoE-/- mice receiving LFA had a dissected abdominal aorta, compared to 0/10 ApoE-/- mice receiving HFA (P>0.05). Neutrophils and macrophages were identified in the adventitia of aortas using transmission electron microscopy and immunohistochemistry. Neutrophil and macrophage infiltration of the infrarenal aorta was lower in ApoE-/- mice on HFA (0.62±0.21 and 5.05±1.41 cells/mm length) than LFA (184.1±193.4 and 15.59±3.32 cells/mm length; P<0.05). The amount of superoxide, Nox2, and MMP-9 in the inflammatory cells were lower in HFA mice compared to LFA mice (superoxide: HFA, 53.4±6.8 arbitrary units (AU)/cell, LFA, 85.1±4.4 AU/cell; Nox2: HFA, 15.8±5.9 AU/cell, LFA, 45.5±6.6 AU/cell; MMP-9: HFA, 25.1±2.7 AU/cell, LFA, 62.0±14.0 AU/cell; P<0.05). The plasma concentration of resolvin D1 was higher in HFA (743±76 pg/ml) compared to LFA mice (457±60 pg/ml; P<0.05). This study showed beneficial effects of a high n-3 PUFA diet in a mouse model of pre-abdominal aortic aneurysm. Animals on the HFA diet were protected against infiltration of inflammatory cells, and production of mediators that are associated with destruction of the aortic wall. Resolvin D1 has been reported to have pro-resolving activity. Future studies will determine if the elevated plasma concentration of resolvin D1 in HFA mice contributed to the protective effect.

AVBS 15: SUPPLEMENTATION OF APOE-/- MICE WITH A HIGH N-3 PUFA DIET PROTECTS AGAINST OXIDATIVE STRESS IN THE ABDOMINAL AORTA


University of New South Wales; ¹University of the Sunshine Coast; ²University of Queensland

AVBS 16: BIOMIMETIC MICROVASCULAR CHIP FOR HIGH THROUGHPUT THERAPEUTIC RESEARCH

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and gelated using a needle (120–300 μm in diameter) as the channel mold. After 30 min of incubation at 37 ºC, the needle was removed and this produced a cylindrical microchannel. SMC (at a density of 1 x 10^6 cells/mL) was loaded into each microchannel from the inlet reservoir via pipetting, and incubated for 20 min (at 37 ºC/ 5% CO2) to accumulate the cells on the gel surface. The seeded cells were cultivated for 2–3 days to reach a confluent state. Following this, EC was loaded using similar procedure (at a density of 2.5 x 10^6 cells/mL) to form a monolayer endothelial lining covering the SMC layers. Optical and confocal microscopy imaging techniques were used to visualise the cell sprouting behaviours that resemble the in vivo functions, luminal cell-ECM and cell-cell interactions, as well as the formation of a three-dimensional vessel lumen that closely simulates the vascular morphology. Conclusion: We successfully demonstrated a simple and robust method to fabricate a tubular, SMC/EC co-culture vascular construct in the PDMS-hosted collagen gel microchannels. The functional microvasculature model, which is transformable into vascular disease-mimicking tissues, will be applicable for high throughput therapeutic and biopharmaceutical investigations.

AVBS 17: A CRITICAL ROLE FOR DESMOGLEIN-2 IN MELANOMA VASCULOGENIC MIMICRY

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Tumour growth and cancer metastasis rely heavily on the ability of cancer cells to gain access to nutrients and oxygen via blood vessels. Apart from the conventional expansion of the tumour vasculature via angiogenesis, studies have also reported the occurrence of vasculogenic mimicry (VM), whereby the cancer cells align and form vessel-like structures themselves. The ability of primary tumours to undergo VM correlates to poor prognosis and increased metastasis in patients, highlighting its potential as a therapeutic target. Desmoglein 2 (DSG2) is a cadherin well described in the formation of epithelial cell-cell tight junctions or desmosomes. We have characterised and detected the frequent expression of DSG2 on melanoma cell lines via both flow cytometry and western blot. In addition to that, we performed immunohistochemical analysis on 150 melanoma biopsies and discovered that DSG2 expression is present in ~35.4% of samples. DSG2 displayed a diffuse membranous distribution in melanoma cells, suggesting a distinct non-desmosomal adhesion role. To examine the potential role of DSG2 in VM, the ability of various DSG2+ melanoma lines, with their DSG2 expression status confirmed by flow cytometry, to spontaneously align and form tubes was assessed by Matrigel assay. We determined that 5/6 (83%) of the DSG2+ cell lines had tube formation capability. More importantly, when DSG2 expression is present in ~35.4% of samples. DSG2 displayed a diffuse membranous distribution in melanoma cells, suggesting a distinct non-desmosomal adhesion role. To examine the potential role of DSG2 in VM, the ability of various DSG2+ melanoma lines, with their DSG2 expression status confirmed by flow cytometry, to spontaneously align and form tubes was assessed by Matrigel assay. We determined that 5/6 (83%) of the DSG2+ cell lines had tube formation capability. More importantly, when DSG2 was selectively knocked down via either siRNAs or a blocking peptide based on the cell adhesion recognition (CAR) domain of DSG2, the ability of DSG2+ cells to form tubes was significantly reduced. Together, these results suggest that DSG2 is an important mediator of VM, and that targeting DSG2 and hence VM can serve as a novel cancer therapeutic approach.

AVBS 18: FXYD1 SILENCING PROMOTES SMOOTH MUSCLE CELL PROLIFERATION

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Backgrounds and Aims: FXYD1 protein has been found to be strongly associated with the activity of sodium potassium pump by regulating the glutathionylation status of its β1 subunit in heart tissues. However its importance in the vasculature has not yet been fully elucidated. In this study, loss of function analysis was used to investigate the role of FXYD1 in the pathogenesis of vascular diseases in both in vitro and in vivo models.

Methods: siRNA was used to silence FXYD1 expression in Human Coronary Artery Smooth Muscle Cells (HCASMCs). Cell proliferation assay was then performed by measuring Edu incorporation into HCASMCs. Src and MAPK phosphorylation status were determined using western blotting. Aortas from both wild type and FXYD1 KO C56/BL6 mice were examined.

Results: FXYD1 silencing increased cell proliferation rate in HCASMCs for more than 50% (p<0.001) compared to scrambled siRNA. Src and Erk1/2 phosphorylation increased 1.5 fold and 2.6 fold respectively. However, the FXYD1 silencing induced cell proliferation was reversed by the addition of Src specific inhibitor- dasatinib. In in vitro model, the average wall to lumen ratio of aorta in FXYD1 KO mice increased by 22% when compared to wild type mice.

Conclusion: These results suggest that FXYD1 plays an important role in regulating smooth muscle cell biology. Silencing of FXYD1 initiated the activation of kinases, which promotes cellular proliferation and ultimately leads to vascular hypertrophy.

AVBS 19: SILENCING OF FXYD1 UNCOUPLS ENOS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Introduction/Aims: FXYD1 has been shown to be a master regulator of the sodium potassium pump activity in the caveolae of heart tissue, protecting the pump from glutathionylation and oxidative inhibition. However, very little is known about its expression and function in the endothelium, particularly in regard to its potential interaction with other caveolae proteins. Given the molecular mechanism of eNOS uncoupling is via glutathionylation, we examined
whether FXYD1 was expressed in endothelial cells, and whether it had a functional partnership with eNOS.

**Methods:** Co-immunoprecipitation was used to study the interaction of FXYD1 and eNOS. GSH antibody was used for immunodetection of eNOS glutathionylation. Silencing of FXYD1 using siRNA followed by DAF staining or spin trap were used to study the effects of FXYD1 on eNOS function.

**Results:** FXYD1 co-immunoprecipitated with eNOS in human umbilical vein endothelial cells (HUVECs). Silencing of FXYD1 resulted in significantly increased eNOS glutathionylation, associated with significantly reduced NO bioavailability under baseline and acetylcholine stimulated conditions (1μM, 20mins).

**Discussion:** Our findings demonstrated a novel functional partnership of FXYD1 with eNOS, protecting this vital enzyme from glutathionylation-mediated uncoupling. This has important implications for our understanding of ROS-signalling in the vasculature.

### AVBS 20: THE POTENTIAL ROLE OF INTERLEUKIN-3 (IL-3) IN BLOOD VESSEL DEVELOPMENT IN BREAST CANCER

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In Australia, breast cancer is the most commonly diagnosed cancer among women and is attributed to 1 in 25 deaths in women from any cause. Richly vascularised tumours are indicative of highly invasive, aggressive breast cancers and have been correlated with poor patient prognosis. Tumour vascularisation can occur through endothelial cell (EC)-dependent mechanisms (angiogenesis and vasculogenesis) or an EC-independent mechanism; vasculogenic mimicry (VM). VM is where tumour cells themselves align into rudimentary vascular networks complete with extracellular matrix. Increasing evidence now indicates interleukin-3 (IL-3) and the alpha chain of its receptor (IL-3R) are involved in several human pathologies, notably cancer where levels of receptor expression correlate clinically with reduced patient survival. Studies suggest that tumour-derived ECs secrete IL-3. IL-3 promotes EC migration and VM in vitro, and stimulates proliferation of endothelial progenitor cells (EPCs). We proposed that IL-3 as a key regulatory factor in breast cancer that mediates vascular development thus tumour progression. We aimed to show this as a unifying mechanism that explains both the contributions from cancer cells and blood vascular cells; namely that breast cancer cells subvert normal developmental processes by (i) recruiting EPCs and (ii) undergoing VM. We have shown IL-3R specific antibodies block VM formation of breast cancer cell lines in vitro. In an in vivo study of breast cancer progression, NOD/SCID mice showed reduced tumour size when treated with IL-3R blocking antibodies. We believe that IL-3 represents an overlooked factor in the progression of breast cancer and that by targeting it with our IL-3R specific antibodies we will develop a new treatment option for breast cancer patients that for the first time attacks both the EC-dependent and VM components of disease progression.

### AVBS 21: TUMOUR NECROSIS FACTOR-RELATED APOPTOSIS INDUCING LIGAND (TRAIL) PROTECTS AGAINST DIET-INDUCED NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN APOE-/− MICE

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Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in developed countries. NAFLD is characterised by the accumulation of triglycerides in the liver, in the absence of excess alcohol consumption. It incorporates a spectrum of liver diseases including steatosis, nonalcoholic steatohepatitis and cirrhosis. There is currently no cure for NAFLD, and treatment options are limited to lifestyle management and anti-diabetic drugs. We have previously shown that tumour necrosis factor-related apoptosis inducing ligand (TRAIL), a TNF cytokine, is protective of atherosclerosis and diabetes in mice. Human patients with NAFLD have elevated circulating TRAIL levels; whether TRAIL plays a pathogenic or protective role in NAFLD is unclear. In this study we placed 6 w old male ApoE-/− and TRAIL-/−ApoE-/− mice on an atherogenic high fat diet for 12 weeks. As expected, TRAIL-/−ApoE-/− mice gained more weight than ApoE-/− mice. They also had significantly higher blood glucose levels and impaired glucose tolerance following a glucose challenge. Further, TRAIL-deficiency in ApoE-/− mice resulted in higher plasma alanine transaminase, a marker of NAFLD in humans. Plasma triglyceride, non-esterified fatty acid and β-hydroxybutyrate levels were also significantly elevated. Importantly, TRAIL-/−ApoE-/− mice displayed increased signs of hepatic steatosis compared to ApoE-/− mice when analysed histologically. Hepatic PPARγ, MCP-1, collagen IV, and MMP2 expression, was also elevated in TRAIL-/−ApoE-/− mice, suggesting TRAIL regulates genes involved in fatty acid synthesis, fibrogenesis and inflammation. This is the first report of a protective role for TRAIL in fatty liver disease. Further work is needed to elucidate whether TRAIL may be used as a potential therapy in the treatment of NAFLD and related conditions.

### AVBS 22: LIMITATIONS IN CLINICAL AND PRECLINICAL RESEARCH ON RENAL ARTERY SYMPATHETIC DENERVATION

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Renal artery sympathetic denervation (RASD) is a standard therapy to lower blood pressure in patients with resistant hypertension in Australia and many other countries. This therapy was first approved as a treatment option for resistant hypertension in 2010. More than 16,000 hypertensive patients have undergone this procedure in the...
past 4 years worldwide. Although a large number of clinical trials on RASD have been conducted and have shown a blood-pressure-lowering effect after RASD, there are major limitations in these trials. These limitations include: (1) a method to verify the completeness of RASD is lacking; (2) office blood pressure measurement has limitations; (3) drug adherence is not ensured; (4) follow-up rates are low; (5) renal artery stenosis is not thoroughly monitored; and (6) long-term effects of RASD are not emphasized. Possible solutions to overcome these limitations in future clinical trials include: (1) research on investigating methods to verify the completeness of renal denervation should be emphasized; (2) ambulatory blood pressure should be used as one of the selection criteria and changes in ambulatory blood pressure should be one of the primary end points; (3) measuring ambulatory blood pressure after witnessed intake of antihypertensive drugs is the best way to ensure drug adherence and should be adopted in future trials; (4) high follow-up rates should be emphasized; (5) all patients after RASD should undergo computerized tomographic angiography—the gold standard method to detect renal artery stenosis; (6) long-term effects of RASD should be emphasized and the crossover design should be discouraged. In addition, preclinical studies are currently limited and need to be emphasized in the future. For example, long-term effects of RASD on cardiovascular disease outcomes are not yet investigated in pre-clinical studies. The sham-controlled, blind and randomized Symplitude HTN-3 trial recently failed to demonstrate a blood-pressure-lowering effect after RASD. This trial has a number of limitations. For example, the success of RASD was not verified. Therefore, future clinical trials need to overcome the current limitations in trial design to investigate the true efficacy and safety of RASD.

AVBS 23: A NOVEL MICRORNA-BASED INHIBITOR, BLOCKMIR CD5-2, NORMALISES THE ANGIOGENIC VASCULATURE AND INHIBITS TUMOUR GROWTH

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Angiogenesis promotes tumour progression and malignancy. In contrast to the normal vasculature, tumour blood vessels are highly abnormal both structurally and functionally. The vessels display a disorganized architecture, poor mural cell coverage, a permeable endothelial cell layer, and the tumour shows hypoxic areas and poor blood perfusion. “Vascular Normalization” is a novel anti-tumour strategy whereby the abnormal angiogenic vessels are returned to a more normal structure and function. This results in an increase in oxygen delivery with potential to improve chemotherapeutic efficacy.

VE-cadherin is a central regulator of endothelial cell junctions and is required for maintaining a restrictive endothelial barrier. Tumour vascular permeability due to disruption, loss or disorganization of the interendothelial junctions can be improved by VE-Cadherin modulation. Our previous studies have identified VE-cadherin as a target for miR-27a. Further we have formulated a novel microRNA-based inhibitor (Blockmir CD5-2), that specifically upregulates the expression of VE-Cadherin and reverse vascular leak through inhibition of miR-27a activity on VE-Cadherin.

In the present study, we have shown that Blockmir CD5-2 inhibits B16F10 melanoma tumour growth and invasion. CD5-2 although having no major effect on vessel density, increases pericyte coverage, decreases tumour vascular permeability and hypoxia, and enhances blood perfusion. All these parameters point to the “normalization” of vessels. Thus, restoration of vascular junctions by Blockmir CD5-2 may be a first-in-class drug to induce tumour vascular normalization and exert anti-tumour effects.

AVBS 24: CATHETER-BASED RADIO-FREQUENCY RENAL DENERVATION REDUCES MONOCYTE ACTIVATION IN HYPERTENSIVE PATIENTS


Background: Up to 50% of all hypertension cases are attributed to over activation of the renal sympathetic nervous system (SNS). Inflammatory, innate immune cells such as monocytes have been shown to play a role in hypertension. We developed a single-chain antibody (MAN-1) that specifically detects monocyte activation by targeting the conformational change of integrin Mac-1 (αmβ2; CD11b/CD18). Catheter-based Radio-Frequency Renal Denervation (RDN) can reduce sympathetic activity in patients with resistant hypertension; however its effect on systemic inflammation is not fully established.

AIM: To investigate whether a reduction in renal SNS activity via RDN modulates monocyte activation and inflammation in hypertensive patients.

Methods: Forty-four patients with hypertension, aged 18–85 years (63 ± 11), underwent the RDN procedure at the Heart Centre in the Alfred Hospital using the radiofrequency Symplicity®-Catheter. Peripheral blood was obtained from patients at baseline, 3 months and 6 months after the procedure. Monocyte activation, monocyte platelet aggregate formation (MPA) and reactive oxygen species (ROS) production were assessed using flow cytometry. Plasma levels of MCP-1 and IL-1β were measured by ELISA. Statistical analyses were conducted using one-way paired ANOVA.

Results: MAN-1 binding to monocytes significantly decreased at 3 (p<0.05) and 6 (p<0.05) months after RDN, indicating a reduction in monocyte activation. Similarly, MPA formation was reduced at both time points (3 months p<0.01; 6 months p=0.052). RDN also lowered plasma levels of MCP-1 (3 months p<0.0001; 6 months p<0.05) and IL-1β (3 months p<0.05) at 3 months, however there were no changes in ROS production.

Conclusion: Our results indicate that a reduction in renal SNS activity via RDN also lowers inflammation in hypertensive patients which provides a unique proof of concept for a direct interaction between nervous system and innate immune system. This may provide an alternate mechanism by which RDN improves cardiovascular outcome in hypertensive patients.
HBP 01: PULSE WAVEFORM ANALYSIS IN PATIENTS WITH ATRIAL FIBRILLATION: REPRODUCIBILITY STUDY WITH THE SPHYGMOCOR SYSTEM

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Background: To date, it has been considered suitable to use pulse wave analysis system such as SphygmoCor only in patients with steady sinus rhythm, where patients with atrial fibrillation (AF) are excluded.

Aim: To test the applicability of the SphygmoCor and its ensemble-averaging process in these AF patients for providing an aortic pressure waveform and its associated indices.

Methods: Seventeen AF patients (11 males, age 83±5 years) were studied in a cardiovascular outpatients setting. Brachial pressures were taken with Korotkov sound method, averaged between two measurements. These values were used to calibrate the ensemble-averaged radial waves measured at the wrist with applanation tonometry. Recordings of the radial pressure were taken over a ten second period on 4–6 occasions. We sought to compare differences in the indices of the aortic pressure waves which were clinically useful: for pressure, pressure from wavefoot to first systolic shoulder (P1), systolic (ASP), augmented (AP), end-systolic pressure (ESP) and pulse pressure (APP); for time intervals, cycle length (CL), heart rate (HR), ejection duration (ED), time to P1 (T1) and time to ASP (T2); for non-dimensional indices, augmentation index (AIx), AIx corrected for averaged heart period (AIx@75), and amplification of pulse height (pulse pressure) between ascending aortic and brachial (PPA).

Results: For all parameters, average values were calculated together with standard deviation (SD) and coefficient of variation (CV = SD÷mean), as follows (mean, SD, CV): P1 (34.9±0.01 mmHg), ASP (118.2±1.00 mmHg), AP (12.3±0.09 mmHg), ESP (104.7±0.01 mmHg), APP (47.3±0.02 mmHg), CL (839.5±0.04 ms), ED (288.7±0.03 ms), T1 (98.1±0.02 ms), T2 (202.7±0.02 ms), AIx (24.6±0.08 %), AIx@75 (24.0±0.09 %), PPA (1.34±0.03 %). The CV were surprisingly small, averaging 0.01 to 0.09 for pressure indices, 0.02 to 0.04 for timing indices, and 0.02 to 0.09 for non-dimensional indices. Such variability was most apparent when the pressure waves were recorded sequentially, with surprisingly similar timing for systole, to peak ejection, and to the peak of pressure.

Conclusion: Results of this study provide a good reason for using pulse wave analysis systems in AF patients. With a single recording, waveforms show far greater similarity than one would expect. However, when multiple waveforms are averaged, the parameters are consistent and reproducible. Therefore the use of pulse wave analysis in AF patients is warranted, at least in stable AF with well controlled HR.

HBP 02: RELIABILITY OF PULSE WAVEFORM ANALYSIS TO MONITOR NITRATE TOLERANCE

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Background: Isosorbide mononitrates (ISMN) have little or no effect on brachial cuff pressures, but substantial effects on aortic pressures and wave contour. Such changes are attributable to dilation of muscular conduit arteries, and reduction of wave reflection from peripheral arterioles. This effect explains therapeutic effects of nitrate, but the effect has never been used to quantify nitrate tolerance.

Aim: To quantify tolerance to ISMN in healthy volunteers through measurement of aortic pressure waveform indices after repeated oral administration of ISMN.

Methods: Eighteen healthy male volunteers (mean age 23±2.5 years) were admitted to Fuwai hospital and studied over three 24-hour periods – baseline (BL, day zero), day 1 – after oral administration of 60 mg ISMN slow release tablet, and at day 6 – after oral administration of the same dose at the same time on days 1–6. The radial artery waveform measured by applanation tonometry and calibrated to brachial cuff pressures. Radial pressure was converted to an aortic pressure wave, and both were ensemble-averaged. Blood levels of ISMN were measured over the 24-hour period on days 1 and 6. Central pressure indices taken over 12 occasions over 24 hours were used to show initial effect of ISMN (at BL), and reduction in nitrate therapeutic effect (tolerance) by comparing indices at day 1 and day 6. Effects of ISMN were studied through changes in aortic pressure between BL and day 1, and tolerance from reduction of these changes between day 1 and day 6. Indices were averaged between 0 to 6 hours after oral administration of ISMN.

Results: ISMN blood levels were similar on days 1 and 6 (475±195 ng/ml vs. 388±194). Brachial cuff pressures showed no significant differences between the three days. However, aortic systolic (ASP) and pulse pressure (APP) together with aortic augmentation index (AIx) decreased significantly between BL and day 1 (ASP 98±2 mmHg vs. 90±1, APP 27±1 mmHg vs. 23±1, AIx 46±9% vs. 22±5), but decrease was less on day 6 (ASP 96±1, APP 25±1, AIx 33±7) compared to day 1 (P<0.005 for all). Amplification of pulse between aorta and brachial artery (PPA) increased on day 1 compared to
**HBP 03: SHOULD ALDOSTERONE SUPPRESSION TESTS BE CONDUCTED DURING A PARTICULAR PHASE OF THE MENSTRUAL CYCLE, AND, IF SO, WHICH PHASE?**

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**Background:** Since levels of renin and aldosterone vary during the menstrual cycle, and are critical criteria for interpretation of aldosterone suppression tests to confirm or exclude primary aldosteronism, it is likely that the outcome will vary depending on the phase of the menstrual cycle. With appropriate information, it will be possible to recommend the best phase of the cycle for diagnostic testing for primary aldosteronism in menstruating women.

**Aim:** To obtain information on the effect of stretching on the levels of renin, aldosterone, progesterone, estradiol, cortisol, LH and FSH during fludrocortisone suppression testing (FST).

**Methods:** In 22 women undergoing FST who experienced regular menstrual cycles, midmorning upright levels of renin (measured as both plasma renin activity and direct renin concentration), aldosterone (measured by mass spectrometry) and cortisol, progesterone, estradiol, LH and FSH (measured by immunoassay) at the conclusion of the 4 day test were compared, relative to the phase of the cycle. Aldosterone levels in both luteal and follicular groups were compared with those in age-matched males.

**Results:** Median (range) levels of progesterone [follicular 1 (1–6) vs. luteal 26 (11–42) nmol/l (P<0.0001)] and aldosterone [600 (222–1600) vs. 254 (18–437) pmol/L (P=0.006)] were both higher in nine women (after one of 10 was excluded with anovulatory cycle) studied during the luteal phase of the cycle than in 12 studied during the follicular phase. All women studied during the luteal phase had a positive FST (day 4 midmorning upright aldosterone >165 pmol/L) and all three with negative FST were studied during the follicular phase. There were no significant differences in other parameters measured except FSH, which was higher (P=0.02) during the follicular phase. Aldosterone was significantly higher in women studied in the luteal (but not follicular) phase compared to men [278 (59–386); P=0.01]

**Conclusion:** The menstrual cycle may affect the outcome of fludrocortisone and other suppression testing used to diagnose primary aldosteronism. Larger patient numbers and preferably restudy of the same patient in both phases by seated saline suppression testing are needed to clarify this question, and to determine the optimum time in the cycle for testing for primary aldosteronism.

**HBP 04: EFFECT OF CYCLIC STRETCH ON ENDOTHELIAL NITRIC OXIDE SYNTHASE AND ASSOCIATED CELL SURVIVAL PATHWAYS IN VASCULAR ENDOTHELIAL CELLS**

Avadhanam BR, Gangoda S, Gupta V, Butlin M, Avolio AP

**Aim:** To determine whether exposure of primary human umbilical vein endothelial cells (HUVECs) to cyclic stretch alters the expression and activity of eNOS and associated survival signaling pathways.

**Methods:** HUVECs (P7) were grown in fibronectin coated silicone chambers at 37°C with 5% CO₂ for approximately 24 hours then subjected to uni-axial cyclic stretch of 1 Hz over 18 hours with stretch magnitude of either 0, 5, 10, 15 or 20% using the ShellPa mechanical stretch system (Menicon Life Sciences, Japan). Percent cell viability was measured using the Countess automated cell counter. eNOS mRNA and eNOS were measured by quantitative RT-PCR and western blotting assays, respectively. The levels and the activity of Akt and GSK 3β proteins were evaluated using western blotting. GAPDH expression was used as an internal control for comparison.

**Results:** In response to the cyclic stretch of varying magnitudes, an increase in the eNOS mRNA at both 5% and 15% CO₂ was observed compared to no (0%) cell stretch (P<0.05; n=3). A further increase in eNOS mRNA was observed when the cells were exposed to 20% cyclic stretch (P<0.05; n=3). In accordance with the changes in eNOS gene transcription in cells, a qualitative increase in the eNOS protein was observed at 20% stretch levels. Similarly, Akt level was found to be slightly increased following 10% and 20% stretch. Interestingly, phosphorylation and level of GSK3β, which is downstream of Akt, was enhanced in cells exposed to higher magnitude of stretching conditions. No significant changes in the cell viability were observed when cells were exposed to either 5% or 20% stretch.

**Conclusions:** Increased eNOS may implicate an enhanced production of the second messenger NO which regulates the vasodilatation in physiological systems. Our study of HUVECs indicate that cyclic stretch is associated with enhanced eNOS expression and upregulation of associated cellular survival signaling pathways. Further studies in animal models will help to correlate these biochemical changes in the context of increased pulse pressures in hypertension.
HBP 05: USE OF RADIAL PRESSURE PULSE FOR ASSESSMENT OF RELATIONSHIP BETWEEN SUBENDOCARDIAL VIABILITY RATIO AND PLASMA B-TYPE NATRIURETIC PEPTIDE IN HEART FAILURE

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Background: The subendocardial viability ratio (SEVR) when computed from the aortic pressure is related to degree of subendocardial myocardial perfusion. In heart failure (HF) cardiac function has increased dependency on myocardial oxygen consumption. Oxygen demand can be estimated from the arterial pressure wave by the systolic tension time index (STI) and oxygen supply from the diastolic time index (DTI). SEVR is computed as the ratio DTI/STI. The severity of HF is also related to plasma levels of B-type natriuretic peptide (BNP). The non-invasive measurement of central aortic pressure from the peripheral (radial/brachial) pressure pulse is based on a generally stable relationship between central and peripheral waveforms.

Aim: To assess the relationship between plasma BNP and SEVR when determined from the radial pressure wave in HF patients, based on the intrinsic relationship between SEVR determined from the peripheral and central aortic pressure waveform.

Methods: Patients (n=17, 7 females) aged 45–82 years (mean 67.5±10.55SD) who were diagnosed with HF according to the New York Heart Association (NYHA) classification (II [n=7]; III [n=7]; IV [n=3]) underwent non-invasive measurement of radial pulse waveform (tonometry), sphygmomanometric blood pressure (BP), left ventricular ejection fraction (LVEF, echocardiography) and plasma BNP concentration (chemiluminescence, Architect ci16200, Integrated System). SEVR (average of at least 10 consecutive pulses) was computed from STI and DTI using ejection duration (ED) determined from the beginning of the pulse to the dicrotic notch of the radial tonometric pulse.

Results: There was an increase in both plasma BNP concentration and SEVR and a decrease in LVEF with NYHA class severity of HF. For the whole cohort there was a significant correlation between SEVR and BNP (pg/ml) (SEVR = -0.0002 BNP + 1.64; r=0.7; P<0.05) for a BNP range of 347–4293 pg/ml. Increasing tertiles of BNP were associated with significant decrease in SEVR (P<0.05) but not heart rate, age, systolic BP or ED. Separate measurements of non-invasive aortic pressure from the peripheral pulse pulse (SphygmoCor, AtCor Medical) confirmed a high correlation (r>0.9) between SEVR from the radial pulse and SEVR from the corresponding derived central aortic pressure.

Conclusions: SEVR computed from the radial pulse has an inverse relationship with plasma BNP in HF. Further studies will aim to assess whether the addition of SEVR as a screening parameter will improve the HF discriminating power of BNP measurement.

HBP 06: AMBULATORY BLOOD PRESSURE MONITORING PARAMETERS AND THE EFFECT OF NIGHT TIME BLOOD PRESSURE MEDICATIONS ON NOCTURNAL DIPPING AND MORNING BLOOD PRESSURE SURGE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background: Ambulatory blood pressure monitoring (ABPM) is the gold standard for assessing blood pressure (BP) in the general population. The prognostic significance of ABPM and the effect of night time BP medications on nocturnal dipping and morning blood pressure surge (MBPS) have been documented in the general population, but less well-defined in chronic kidney disease (CKD) patients.

Aim: To outline ABPM characteristics in different CKD stages and the effects of night time medications on BP control, nocturnal dipping and MBPS.

Method: CKD (n=157) patients underwent 24-hour ABPM monitoring (Spacelabs ABPM monitor). 24-hour awake and asleep BP averages and percentage nocturnal dipping were calculated from the ABPM data. A BP fall of ≤ 10% during sleep defined subjects as non-dippers. MBPS was calculated as the difference between morning BP (average of 4 consecutive readings over 2 hours immediately after awakening) and nadir during sleep (average of at least 2 readings around the lowest BP reading during sleep). The timing of antihypertensive medication use was recorded. The stages of CKD were grouped as mild, moderate and advanced CKD (34%, 56%, and 68%, respectively in mild, moderate and severe CKD, (P<0.05). Administration of antihypertensive medications at bedtime did not significantly influence the dipping pattern of patients across different stages of CKD (no night time medication vs. night time medication, 8.6±1.0 vs 9.0±1.0 %). A subgroup analysis of CKD patients (n=105) revealed no significant reduction in MBPS with use of night time BP medications (no night time medication vs. night time medication, 19.8±5.3 vs. 20.6±2.0%).

Conclusion: Higher prevalence of non-dipping status was observed with advancing stages of CKD. In contrast to published studies, the use of night time medications did not influence dipping status or magnitude of MBPS in CKD.
HBP 07 CONTRASTING EFFECTS OF PRENATAL LIFE STRESS ON BLOOD PRESSURE AND BMI IN YOUNG ADULTS

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Background: Various environmental stressors in pregnancy have been reported to affect high blood pressure (BP) in adult offspring. However few studies have examined the effect of prenatal maternal psychological stress on offspring BP and BMI in early adulthood.

Aim: To test the hypotheses that prenatal life stress will be associated with higher BP and higher BMI in a young adult population.

Methods: In 957 Raine cohort participants regression analyses were used to examine the association between the count of prenatal life stress events experienced during pregnancy and offspring BP and body mass index (BMI) at age 20.

Results: Prenatal life stress was positively associated with offspring BMI but inversely associated with systolic blood pressure (SBP). After adjustment for confounders each additional prenatal life stress event reduced offspring SBP by 0.66 mmHg (P=0.013) in those with an average BMI and lowered the odds of systolic (pre)hypertension by 17% (OR=0.83; P=0.008). The inverse relationship between prenatal life stress and adult SBP was stronger in offspring with higher BMI. On the other hand, each unit increase in prenatal life stress score predicted a BMI increase of 0.37 kg/m² (P=0.022).

Conclusions: This study has shown that maternal stress in pregnancy is significantly associated with BMI, but contrary to our hypothesis predicted lower resting systolic BP and lower odds of systolic (pre)hypertension in young adult offspring. The effect of prenatal life stress on BP was accentuated by a higher BMI. Fetal programming events as a result of prenatal stress may underpin these relationships.

HBP 08: VALIDATION OF AMBULATORY CUFF-BASED DEVICES FOR CENTRAL AORTIC BLOOD PRESSURE AND ARTERIAL STIFFNESS MEASUREMENT

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Background: As the utility of non-invasive measurement of central aortic blood pressure parameters grows, the logical next step is for out-of-clinic measurement of aortic blood pressure parameters. Recently, ambulatory blood pressure monitors (ABPMs) have come on to the market with this ability.

Aim: To investigate the accuracy of two ABPM devices that estimate central aortic blood pressure (BPLab* and AtCor/SunTech Oscar 2) by comparing them in-clinic to existing validated devices, the Sphygmocor XCEL (cuff-based) and tonometer-based devices.

Methods: 45 subjects were recruited for the study, with 1 subject being excluded due to arrhythmia. The remaining subjects were 46±17 years old (30 male) with brachial systolic pressure ranging from 104 to 153 mmHg, diastolic pressures from 63–106 mmHg, heart rate from 48–94 bpm. Measurements were taken in triplicate with each device and averaged for repeated-measures statistical comparison of calculated central aortic systolic, diastolic, and augmentation index (AIx) between devices. Devices were also compared by Bland-Altman representation. Calculated aortic systolic pressure was 4.1±3.3 mmHg higher in the Oscar 2 device than the tonometer-based device (tonometer-based 112±2, XCEL 113±2, Oscar 2 116±2 mmHg; P=0.20). Aortic diastolic pressures were consistent between devices (tonometer-based 79±1, XCEL 80±1, Oscar 2 79±1 mmHg; P=0.067). Aortic Alx was greater in the XCEL, and greater still in the Oscar 2 device compared to the tonometer-based device (tonometer-based 13±2%, XCEL 18±2%, Oscar 2 25±2%; P=0.003). There was little bias seen in the Oscar 2 device compared to the tonometer-based device for either aortic systolic (slope=0.92, intercept=12.7 mmHg, R²=0.92, P<0.001) or aortic diastolic (slope=1.00, intercept=0.3 mmHg, R²=0.99, P<0.001). However, Alx had a slope between devices less than unity (slope=0.74, intercept=15.7%, R²=0.36, P<0.001). Taking international guidelines for brachial blood pressure measurement in the absence of guidelines for central aortic blood pressure calculation, the Oscar 2 device has a Grade A rating. However, calculated augmentation index was considerably different between devices. (BPLab data has been collected, but the analysis is currently unavailable due to a software license issue that will be resolved before the conference date.)

Conclusions: Our findings suggest that the cuff-based ABPM calculation of aortic systolic and diastolic pressure out of clinic settings appears feasible. However, the calculation of waveform shape parameters, such as augmentation index, requires further investigation.

HBP 09: MUSCLE FITNESS AND BLOOD PRESSURE IN CHILDREN THROUGH TO LATE ADOLESCENCE

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Background: Cardiorespiratory fitness has been shown to reduce mortality rates and attenuate health risks associated with increased adiposity. Muscle strength is an important aspect of physical fitness and low levels of muscle strength are associated with morbidity and mortality outcomes in adults. Muscular mass has been inversely associated with onset of insulin resistance, Type 2 diabetes and high blood pressure (BP) in adults. However, there is a lack of data examining the relationships between muscle strength with BP in childhood and adolescence.
Aim: To examine the association between hand grip strength, back endurance and BP from childhood through to adolescence.

Methods: The study included 1916 participants from the Western Australian Pregnancy Cohort (Raine) Study examined at ages 10, 14 and 17 years. Hierarchical linear mixed model analyses were performed.

Results: The mean hand grip strength in males at 10, 14 and 17 years was 30.5 kg, 51.8 kg and 65.5 kg, respectively, and increased over time (P<0.001). Systolic BP increased significantly from 10 years (106.5 mm Hg) to 17 years (113.2 mm Hg (P<0.001). Hand grip strength was significantly associated with a higher systolic BP over the 10–17 year age span in both males and females (P≤0.001) in regression models that adjusted for gender and BMI, but the strength of association was attenuated over time. An increase in hand grip strength of 10 kg was associated with a higher systolic BP of 1.6 mm Hg at 10 years and 0.07 mm Hg at 17 years. Back endurance was significantly associated with a higher systolic BP at 14 years (P<0.05) and this association was maintained from 14 to 17 years. Every 100 second increase in back endurance was associated with a 1 mm Hg higher systolic BP.

Conclusions: This the first study to examine the relationship between muscle strength and systolic BP from childhood through to late adolescence. The positive association is contrary to that seen in adult populations. However, the attenuation by age 17 suggests a growth-related effect. Hence further studies as the population moves into adult life will be of interest. The underlying mechanism warrants further investigation and the results need to be verified in other young populations.
Conclusions: This study provides early evidence that acute administration of hAECs improves outcome following ischemic stroke. This improvement may be in part via prevention of brain injury but also via limiting stroke-induced systemic immunosuppression. However, further investigations are required to determine whether these improvements evoked by hAECs translate to a reduction in bacterial infections.

HBP 12: HIGH BLOOD PRESSURE AND CYCLIC STRETCH ALTER CEREBRAL AMYLOID DEPOSITION AND ENDOTHelial FUNCTION

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Background: Amyloid β (Aβ) deposition is a hallmark of Alzheimer’s disease (AD). Increased pulsatility, endothelial dysfunction (ED) and inflammation, as indicators of vascular stiffness, are associated with (AD). Additionally, vascular stiffness is linked to hypertension, a risk factor for AD.

Aim: To determine effects of high blood pressure (BP) on cerebral Aβ deposition in rodent models – spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats – and investigate effects of pulsatile cyclic stretch (CS) on expression of amyloid precursor protein (APP), endothelial nitric oxide synthase (eNOS) and intercellular cell adhesion molecule-1 (ICAM-1) in human cerebral microvascular endothelial cells.

Methods: Hippocampal (HC) and frontal cortex (FC) regions of SHR and WKY rats were analysed using western blotting (WB) to determine effects of BP on cerebral Aβ deposition. hCMEC were subjected to 5%, 10% or 20 % CS compared to control (0% CS) to evaluate pulsatility, ED and inflammation using Western blot and/or quantitative RT-PCR.

Results: Aβ oligomerization was increased in SHR compared to WKY in HC (P<0.01) and FC (P<0.001). APP mRNA level was increased at 5%, was decreased at 20% while eNOS was decreased at both (P<0.0001). APP and ICAM-1 protein levels were dose-dependently increased at 5% and 10% CS (P<0.01) and decreased at 20% CS. eNOS protein levels were decreased at all CS (P<0.0001).

Conclusions: Our results suggest that high BP and CS, respectively, alter the processing and expression of cerebral APP. Prolonged CS may induce ED by increasing ICAM-1, thereby mitigating eNOS expression. Findings mechanistically support the association of elevated pulsatility and arterial stiffness with AD.

HBP 13: DOES CONCOMITANT AUTONOMOUS ADRENAL CORTISOL OVERPRODUCTION HAVE THE POTENTIAL TO CONFOUND THE INTERPRETATION OF ADRENAL VENOUS SAMPLING IN PRIMARY ALDOSTERONISM?

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Background: Primary aldosteronism (PA) is a common secondary form of hypertension, with unilateral forms potentially curable by adrenalectomy (ADX). Lateralization of aldosterone (aldo) production by adrenal venous sampling (AVS) is required for ADX. Criteria used are (1) successful cannulation, defined by an adrenal/peripheral cortisol gradient ≥3, (2) aldo/cortisol ratios (ACR) on the affected side ≥2 times peripheral and (3) ACR on the contralateral (CL) side ≤ peripheral (CL suppression). We encountered a patient with an aldosterone-producing adenoma (APA) that co-secreted cortisol and in whom AVS failed to lateralize despite subsequent cure of PA following APA removal. We hypothesized that autonomous cortisol secretion confounded the AVS.

Aim: To test the hypothesis that autonomous cortisol secretion confounded AVS by examining hormone levels in patients with isolated cortisol-producing adenoma (CPA) who underwent AVS.

Methods: Eight CPA patients had AVS and Cushing syndrome (n=6; 2 “subclinical”), unsuppressable cortisol by dexamethasone (n=8), suppressed ACTH (n=6; 2 unavailable), unilateral lesion on imaging (n=8) and clinical resolution of hypercortisolism post ADX (n=7; 1 unavailable). All had normal plasma aldo/renin ratios. AVS results were assessed using diagnostic criteria used for lateralization of aldo in PA.

Results: Cortisol levels were higher on the ipsilateral (IL) than the CL side of the tumor in all (median 6.7 [range 2.4–27.2] fold; P=0.012), consistent with CPA. By our usual criteria, catheter placement would have been labeled as inadequate CL to the CPA in 6 (75%), presumably because of suppressed cortisol production, despite adrenal vein aldo levels being markedly higher than peripheral (median 41.6 [range 7.2–511] fold; P<0.001), suggesting successful cannulation. In all patients, adrenal vein ACR CL to the CPA were at least 2-fold higher than peripheral, but in only 3 patients IL to the CPA (median fold difference CL 44.5 [range 6.0–109] vs IL 1.65 [1.0–23.0]; P=0.017). ACR were higher CL vs IL in 7 (88%); median 6.0 [range 2.0–76] fold) and similar (19 vs. 23) in the remaining patient.

Conclusion: In patients with PA, concurrent autonomous unilateral hypersecretion of cortisol might potentially confound the accuracy of AVS by increasing cortisol levels (reducing ACR) on the side of the CPA, while reducing cortisol levels (increasing ACR and suggesting failed cannulation) on the CL side. Failed lateralization could result in misclassification of AVS. In such patients, use of an alternative (e.g., metadrenaline) to assess success of cannulation and lateralization should be considered.
HBP 15: CONCOMITANT AUTONOMOUS CORTISOL SECRETION BY ALDOSTERONE-PRODUCING ADENOMA: THE UTILITY OF PLASMA METADRENALINE TO ASSESS LATERALIZATION DURING ADRENAL VENOUS SAMPLING

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Background: Primary aldosteronism (PA) is the most common secondary endocrine form of hypertension. Patients with unilateral forms, potentially curable by unilateral adrenalectomy, can be identified by adrenal venous sampling (AVS). AVS criteria for lateralization are (1) adrenal venous aldosterone (aldo)/cortisol (A/F) ratios on the affected side ≥2.0 times peripheral and (2) A/F ratios on the other side ≤ peripheral (contralateral suppression). Concurrent autonomous cortisol production by APA is increasingly recognized. In these patients, there is the potential for confounding of AVS results since cortisol levels are used to correct for differences in dilution of adrenal with non-adrenal venous blood as per the criteria above.

Aim: To describe a case where metadrenaline was measured during AVS to successfully circumvent the problem described above.

Case presentation: A 55 year old hypertensive male had raised plasma aldo/rein ratios suggesting PA which was confirmed by fludrocortisone suppression testing. Failure of plasma cortisol to suppress overnight following either 1 mg or 3 mg dexamethasone and persistently suppressed ACTH in the absence of Cushingoid clinical features were consistent with subclinical adrenal Cushing syndrome. AVS was performed with measurement of metadrenaline to avoid misinterpretation due to hypercortisolism. Comparison of adrenal and peripheral A/F ratios (Left 5.7 vs. Peripheral 1.0; Right 1.7 vs. Peripheral 1.0) yielded results consistent with bilateral aldo production, with the left gland dominant but without contralateral suppression. However, using aldo/metadrenaline ratios (Left 0.35 vs. Peripheral 0.09; Right 0.05 vs. Peripheral 0.09), aldo production clearly lateralized to the left, with good contralateral suppression. The patient underwent left laparoscopic adrenalectomy with peri-operative glucocorticoid supplementation to prevent adrenal insufficiency. Pathological examination revealed an adrenal cortical adenoma. Hypertension improved and cure of PA and hypercortisolism were confirmed by negative post-operative fludrocortisone suppression test and overnight 1 mg dexamethasone suppression test.

Conclusion: Routine dexamethasone suppression testing in patients with PA permits detection of concurrent hypercortisolism which can confound AVS results and cause unilateral PA to be misdiagnosed as bilateral with patients thereby denied potentially curative surgical treatment. In such patients, measurement of plasma metadrenaline during AVS may overcome this issue.
HBP 16: POSSIBLE COMPENSATIVE ACTIVATION OF RAS FOLLOWING RENAL DENERVATION REDUCED BLOOD PRESSURE IN HYPERTENSIVE SCHLAGER MICE

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Background: Hypertensive Schlager mice (BPH/2J) are hypertensive due to an exaggerated contribution of the sympathetic nervous system (SNS). Compared to their normotensive counterparts (BPN/3J), BPH/2J had a greater renal sympathetic innervation and an increase in renin-angiotensin-system (RAS) activity. Thus, we hypothesized that an exaggerated sympathetic renal activity was contributing to elevate blood pressure in BPH/2J.

Aim: To determine the effect of bilateral renal denervation (Rx) on the RAS and SNS activities and cardiovascular parameters of BPH/2J and BPN/3J mice.

Methods: Blood pressure (BP) was measured via pre-implanted radiotelemetry probes before and 3 weeks after Rx (10% phenol) or sham surgery. The BP response to enalaprilat and pentolinium injections was measured 2 weeks after renal surgery to determine RAS and SNS contribution to BP respectively. Kidneys were collected following the final recording to measure noradrenaline content.

Results: Rx reduced BP by 8.0±2.2mmHg in BPH/2J mice over the 3 weeks of the experiment but had no effect in BPN/3J. Following Rx, enalapril decreased BP in BPH/2J mice compared to sham group but had no effect in BPN/3J. These results suggested a greater RAS contribution to maintaining BP in BPH/2J mice after Rx. Conversely, response to pentolinium was greater in BPN/3J mice following Rx compared with the sham group, indicating a greater SNS activity in this group, whereas pentolinium had no effect in BPH/2J mice. Noradrenaline content was both reduced in BPH/2J and BPN/3J mice compared to sham procedure, confirming Rx.

Conclusion: From these findings, we suggest that renal sympathetic innervation is essential in maintaining hypertension in BPH/2J mice. The main effect of Rx that occurred equally in BPN/3J and BPH/2J mice was to reduce the basal RAS and SNS independent contribution to BP. In BPN/3J, BP was maintained by a compensative activation of SNS whereas there was an increase of RAS contribution in BPH/2J. Effectiveness of Rx in reduction of BP may be dependant of the form of hypertension, whether it involves a neuronal or a renal mechanism.

HBP 17: THE ROLE OF microRNA-181a IN MEDIATING THE CONTRIBUTION OF THE RENIN-ANGIOTENSIN SYSTEM TO HYPERTENSION IN BPH/2J MICE

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Background: BPH/2J mice are a genetic model of hypertension driven by greater activity of the sympathetic nervous system (SNS) and renin-angiotensin-system (RAS). During the dark period of the 24-hour light cycle when hypertension is at its greatest, BPH/2J mice display enhanced renal renin mRNA possibly related to lower levels of microRNA-181a, which is a negative regulator of renin mRNA.

Aim: To determine whether lower renal miR-181a abundance contributes to elevated RAS activity and hypertension in BPH/2J mice.

Methods: BPH/2J mice (n=6) were administered a miR-181a mimic (mirVana, 1, 5, 10 and 25nmol i.v) using an in vivo kidney specific transfection reagent and compared with untreated normotensive BPN/3J and BPH/2J mice (n=6–8). Blood pressure (BP) was measured before and for 2 days after mimic treatment via pre-implanted radiotelemetry probes. The BP response to ACE inhibition (enalaprilat) and ganglion blockade (pentolinium) was determined during the dark period ~26 hours after a 25 nmol dose and kidney tissue was collected at ~50 hours.

Results: The 25 nmol dose of the miR-181a mimic caused a 4.0±1.4 mmHg reduction from baseline in diastolic BP during the dark period (P<0.01), whilst the 1, 5 and 10 nmol doses had no detectable effect (P>0.27). Renal renin mRNA abundance in mice treated with the miR-181a mimic was 0.87±0.1, which was lower than untreated BPH/2J mice (1.5±0.2; P=0.02) and comparable with untreated normotensive BPN/3J control mice (0.95±0.1; P=0.93), suggesting that the mimic effectively inhibited renin mRNA in vivo. Furthermore the depressor response to enalaprilat in untreated BPH/2J mice was abolished in BPH/2J mice treated with the mimic (~11.2±2 mmHg vs. 1.5±3 mmHg respectively; P<0.001), suggesting that the mimic reduced the RAS contribution to BP maintenance. The peak depressor response to pentolinium following enalaprilat pre-treatment was comparable between untreated and mimic treated BPH/2J mice (~58±3 vs. -50±3 mmHg; P=0.08), suggesting that the mimic does not overtly affect the SNS contribution to BP in BPH/2J mice.

Conclusion: These findings provide the first in vivo evidence that low miR-181a levels contribute to greater activity of the RAS and hypertension in BPH/2J mice.
HBP 18: OBESITY LIMITS THE NORMAL CARDIAC ADAPTATIONS OF PREGNANCY

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Background: Obesity is associated with cardiac disease and dysfunction in non-pregnant individuals, and with poor maternal and fetal outcomes during pregnancy.

Aim: To examine the impact of obesity on the normal cardiac adaptations of pregnancy and fetal outcomes.

Methods: 4 week old female C57BL6J mice were fed control (7% fat, 3.85 kCal/g) or high fat (23.5% fat, 4.54 kCal/g) chow for 10 weeks and glucose tolerance tests (i.p.) were performed. Cardiac structure and function were assessed by double-gated cine MRI (9.4Tesla MR System) prior to mating and at GA14. Fetal and placental tissues were obtained at GA18.5.

Results: High fat feeding generated obese mice with bodyweights 47% greater than controls (33.3±0.6, 22.7±0.2 g; P<0.001). Obese mice had significantly higher fasting blood glucose compared to control mice (9.0±0.25, 6.9±0.37 mmol/L; P<0.001) and were glucose intolerant (P<0.001). Obese mice had significantly greater left ventricle mass (LVM; 67.5±2.1, 58.7±0.8 mg; P<0.01) and cardiac output (CO; 18.3±0.6, 15.6±0.9 ml/min; P<0.05) compared to control mice. Both LVM and CO increased with pregnancy, but the effects were blunted in obese mice (P<0.001; P<0.01). Post-hoc analysis demonstrated that while the pregnancy-induced increases in LVM (~26%) and CO (~25%) for control mice were profound and significant (P<0.001), the increases in LVM (~6%) and CO (~7%) of obese mice with pregnancy were not significant. Indeed at GA14 the values for LVM and CO were no longer different between obese and control mice. The changes in CO with pregnancy were reflected in changes in stroke volume, with only control mice showing a significant increase with pregnancy. The increase in stroke volume with pregnancy in control mice was due to increases in end-diastolic volume. Neither obesity nor pregnancy impacted ejection fraction. Litter size at GA18.5 was not different between obese and control dams (7.0±1, 7.8±0.7). However, obese dams had significantly greater resorptions and thus significantly fewer viable pups (4.0±0.8, 7.5±0.9; P<0.05). Male and female pups from obese dams (1.01±0.02, 0.98±0.02) had significantly lower bodyweights than male and female pups from control dams (0.83±0.04, 0.85±0.04; P<0.01), higher placental weights (P<0.01) and thus lower fetal:placenta ratios (P<0.01).

Conclusion: Diet-induced obesity limits the normal pregnancy-induced increase in CO. Our data suggest that obesity may compromise establishment of effective fetal supply and explain the high rate of miscarriage, stillbirth and small for gestational age babies in obese women.

HBP 19: ARTERIAL HYPERTENSION IN ASTANA CITY, KAZAKHSTAN. A PILOT STUDY

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Background:

Aim: To assess the prevalence, awareness, treatment, and control of arterial hypertension and factors associated with these indices in a population sample of Astana, the new capital city of Kazakhstan.

Methods: This was a cross-sectional study of subjects registered in 8 outpatient polyclinics in Astana, Kazakhstan. Participants comprised a total of 497 adults (response rate 56%) aged 50–75 years randomly selected from registers of the polyclinics. Hypertension was defined as a mean systolic and/or diastolic blood pressure of ≥140/90 mmHg and/or anti-hypertensive medication use during the previous 2 weeks. Awareness and treatment were based on self-report. Hypertension control was defined as blood pressure <140/90 mmHg among hypertensive subjects.

Results: The overall prevalence of hypertension was 70%. Among hypertensive subjects, 91% were aware of their condition, 77% were taking anti-hypertensive medications, and 34% had blood pressure controlled (<140/90 mm Hg). The prevalence of hypertension and its awareness, treatment and control was more common in women, among persons aged 60 years or more and (except control) among those with high body mass index. None of several available socioeconomic measures was associated with any of the hypertension indices.

Conclusions: The levels of awareness, treatment and control of hypertension in the study group were higher than in most Eastern European and Central Asian populations with available data, most likely reflecting high education and large proportion of civil servants in the new capital city. However, even in this relatively privileged population, the rates of successful control of hypertension were modest.

HBP 20: NORADRENALINE THERAPY IN SEPTIC SHOCK: EFFECTS ON BLOOD PRESSURE AND INTRA-RENAL PERFUSION AND OXYGENATION IN AN OVINE MODEL OF HYPTOTENSIVE SEPSIS

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Background: Previously, we have shown that hypotensive sepsis in sheep is associated with renal hyperemia, but with a progressive decline in renal function. It is likely that reduced filtration and the subsequent decrease in
Melbourne, Victoria, Australia; HR (~90%) and RBF (~70%) and oliguria. At 24 h of
infused intravenously from the 25th-30th hour of sepsis (n=5).

**Results:** Prior to treatment with NA, sepsis was characterized by hypotension (~10 mmHg reduction), along with elevations in
HR (~90%) and RBF (~70%) and oliguria. At 24 h of *E. coli* infusion, cortical tissue perfusion (1335±245 to 1599±325 BPU) and PO₂
(31.4±2.3 to 36.6±3.2 mmHg) were increased, but medullary perfusion (1477±52 to 852±226 BPU) and oxygenation (31.1±0.9 to
19.3±2.9 mmHg) were reduced. Administration of NA restored MAP and UF, but had no effects on HR and RBF. During NA
treatment, cortical perfusion and oxygenation remained unchanged, but there was a further reduction in medullary perfusion (to
707±202 BPU) and oxygenation (14.5±1.9 mmHg).

**Conclusions:** During hypotensive sepsis, renal hyperemia was associated with a reduction in medullary perfusion and oxygenation
that may play a pivotal role in development of AKI. Administration of NE reversed sepsis-induced hypotension and oliguria, but
resulted in a further decline in medullary perfusion and oxygenation. Thus, although NA treatment can restore blood pressure and
renal function during sepsis, it exacerbates medullary hypoxia, which may exacerbate kidney injury in the longer term.

**HBP 22: IMPROVED CARDIAC REPAIR POST-MYOCARDIAL INFARCTION USING NOVEL HUMAN CARDIAC RESIDENT STEM CELLS**

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**Background:** Cardiac stem cells hold much promise for treatment of heart disease, but this remains a nascent field. We have
recently identified a novel population of human cardiac resident stem cells (CRSCs), which are positive for W8B2 antigen. W8B2+
CRSCs can self-renew and are highly clonogenic.

**Aim:** To characterize W8B2+ CRSCs.

**Methods:** W8B2+ CRSCs were isolated from adult human atrial appendages and studied by various techniques.

**Results:** Immunophenotyping showed that W8B2+ CRSCs expressed mesenchymal-related antigens but not hematopoietic- or
endothelial-related antigens. In the resting state, W8B2+ CRSCs expressed HLA-ABC but not HLA-DR antigen. Regarding cardiac
genotag marker, W8B2+ cells express GATA4, HAND2, MEF2C and TBX5 but lack expression of c-Kit, Sca-1, NKX2.5, PDGFRα,
ISL1, and Wilm’s tumor gene-1. This profile is distinct from currently known CRSCs found in the adult human heart, and is further
supported by RNA sequencing analysis which revealed many transcripts that were differentially expressed between W8B2+ and
c-Kit+ CRSC populations. W8B2+ CRSCs were found to secrete many cytokines implicated in angiogenesis, chemotaxis, inflammation,
extracellular matrix remodelling, cell growth and survival. In addition, W8B2+ CRSCs can differentiate into cardiogenic cells which
were responsive to electrical stimulation, as well as into endothelial and smooth muscle cells, and can undergo adipogenesis,
osteogenesis and chondrogenesis. When implanted as cell sheets into an *in vivo* tissue engineering chamber in rats, W8B2+
CRSCs significantly increased intrinsic vascularization of the engineered tissue constructs. Intramyocardial transplantation of
human W8B2+ CRSCs into immunocompromised rats one week after myocardial infarction significantly improved cardiac function
(~40% improvement in ejection fraction) and reduced fibrotic scar tissue. Hearts treated with W8B2+ CRSCs showed less adverse
remodelling of the left ventricle, have greater number of proliferating cardiomyocytes (Ki67+cTnT+ cells) in the remote region,
higher myocardial vascular density, and higher degree of CD163+ cells (a marker for M2 macrophages) infiltration in the border
and scar regions.

**Conclusions:** W8B2+ CRSCs are distinct from currently known CRSCs found in human hearts, and may be an ideal cell source for
tissue engineering to treat ischemic heart disease.

**HBP 23: INTERRELATIONSHIPS BETWEEN CIRCULATING AND URINARY COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM IN INDIGENOUS PREGNANT WOMEN**

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**Background:** The renin-angiotensin system (RAS) is activated in pregnancy. In addition, activation of an
intra-renal RAS might occur to offset the effects of the high glomerular filtration rate and progesterone.

**Aim:** To study gestational changes and interrelationships between circulating and urinary RAS components in
Indigenous Australian women.
HBP 24: KIDNEY AND CIRCULATING microRNA miR-181a IS ASSOCIATED WITH RENIN AND BLOOD PRESSURE IN HUMANS

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Background: MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression by destabilizing mRNAs. We have shown previously that the microRNA miR-181a is able to bind to and regulate the levels of human renin mRNA in transfected kidney cells.

Aims: To determine (i) intrarenal localization of miR-181a and renin, (ii) the association of kidney and serum miR-181a and renin with blood pressure (BP), and (iii) the signatures of miR-181a on the human renal transcriptome.

Methods: The experiments were conducted in 200 European individuals with available kidney and serum, and validated in an independent cohort of 199 serum samples. Real-time PCR was used to measure circulating and renal miR-181a and renal renin mRNA. Serum renin was measured by immunoradiometric assay. In situ hybridization for localization of miR-181a and immunohistochemistry for renin were performed in human kidney sections. Sixty-nine renal transcriptomes characterized by next-generation RNA sequencing were used to assess miR-181a stratified co-expression.

Results: After adjusting for clinical and laboratory variables, there was a negative association between the expression of miR-181a and renin in human kidneys (β=–0.35, SE=0.16, P=0.028). miR-181a and renin mRNA were co-expressed in both renal cortex and medulla. The strongest co-localization was in the tubular epithelium of the distal nephron, most likely the collecting ducts. Serum levels of miR-181a were strongly correlated with its renal levels (β=0.52, SE=0.11, P<0.001). There was a strong association between BP and both renal and serum levels of miR-181a – higher levels of miR-181a were associated with higher systolic and diastolic BP (DBP). The most significant association was between serum miR-181a levels and DBP in those not on BP lowering therapy – each unit increase in miR-181a was associated with an elevation in DBP of 2.5 mmHg (P<0.001). Serum renin was also positively associated with BP. The association between serum miR-181a and BP was, however, independent of serum renin levels. The miR-181a-stratified gene set enrichment analysis of renal transcriptomes revealed down-regulation of mitochondrial function pathways and increased expression of adaptive immunity and inflammation signalling cascades in the kidney

Conclusions: miR-181a is associated with both expression of renin mRNA in the kidney and systemic BP in an independent manner. miR-181a was also associated with the down-regulation of mitochondrial respiratory function pathways combined with activation of adaptive immunity and pro-inflammatory phenotype in the kidney, and these pathways may explain miR-181a association with elevation in BP.
HBP 25: GENETIC VARIATION IN THE RAPTOR GENE IS ASSOCIATED WITH OVERWEIGHT BUT NOT HYPERTENSION IN AMERICAN MEN OF JAPANESE ANCESTRY

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Background: The mechanistic target of rapamycin (mTOR) pathway is pivotal for cell growth and has been implicated in aging, cardiovascular disease, obesity, diabetes and cancer. mTOR signalling is involved in cardiac leptin-mediated cardiac hypertrophy and fibrosis associated with obesity. mTOR is a key component of two multiprotein complexes, mTOR complex 1 and mTOR complex 2. mTOR complex 1 is pro-growth and contains a unique protein, raptor.

Aim: To test, for the first time, whether genetic variation across the raptor gene (\textit{RPTOR}) is associated with overweight/obesity, essential hypertension (EHT) and isolated systolic hypertension (ISH).

Methods: We genotyped 61 common (allele frequency ≥ 0.1) tagging single nucleotide polymorphisms (SNPs) that captured most of the genetic variation across \textit{RPTOR} in 374 subjects of normal lifespan and 439 subjects with a lifespan exceeding 95 years. Subjects were drawn from the Honolulu Heart Program, a homogeneous population of American men of Japanese ancestry, well characterized for phenotypes relevant to conditions of aging. Hypertension status was ascertained when subjects were 45–68 years old. Statistical evaluation was performed by contingency table analysis, logistic regression and recursive partitioning (RP), which is regarded as amongst the most powerful methods for statistical analysis of large complex sets of genetic information.

Results: After analysis of \textit{RPTOR} genotypes by each statistical approach we found no significant association between genetic variation in \textit{RPTOR} and either EHT or ISH. For EHT, RP revealed that even the most predictive SNPs (rs4969322 and rs4890052) provided little contribution to correctly assigning individuals to EHT or NT (\(P=0.22\) by Z test). In the case of ISH, RP revealed that only one SNP (rs2589118) made a noticeable contribution, and that this was no better than the contribution from the weakest laboratory/examination variable (overweight/obesity). In contrast, for overweight/obesity, the RP model revealed that \textit{RPTOR} SNPs significantly enhanced the predictive capacity of the model (\(P=0.008\) by one-tailed Z test).

Conclusion: Genetic variation across RPTOR is associated with overweight/obesity, but not EHT or ISH, in American men of Japanese ancestry. (\textit{Am J Hypertens} Epub ahead of print 22 Sep 2014)

HBP 26: VASCULAR ENDOTHELIAL CX40 CONTRIBUTES TO ACTIVITY-DEPENDENT BLOOD PRESSURE REGULATION

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Background: The role of the gap junction (GJ) protein connexion 40 (Cx40) in renin-dependent regulation of blood pressure is well established. However, the contribution of vascular endothelial Cx40 to blood pressure control is less well understood as previous attempts to isolate the function of endothelial Cx40 by gene deletion have been complicated by coordinate changes in Cx37. We have therefore created a transgenic mouse strain (Cx40T152ATg) expressing a mutant Cx40, Cx40T152A, together with native Cx40, under control of the endothelial cell-specific Tie2 promoter. Endothelial Cx40 is essential for ascending vasodilation, a major contributor to the mechanism that increases blood flow and decreases peripheral resistance during exercise by coordinating vasodilation of microcirculatory networks with upstream supply vessels. We therefore hypothesised that disruption of endothelial Cx40 function would lead to dysregulation of blood pressure during activity.

Aim: To test whether disruption of endothelial Cx40 function would lead to dysregulation of blood pressure during activity.

Methods: The impact of Cx40 T152A on electrical and chemical conductance was assessed using \\textit{Xenopus} oocytes and mouse coronary endothelial cells in vitro. Immunohistochemistry was used to determine transgene expression and its effect on endogenous Cx37. Pressure myography was used to assess myoendothelial GJ coupling in mesenteric arteries in vitro and ascending conducted vasodilation in response to acetylcholine was used to assess endothelial GJ coupling in cremaster arterioles in vivo. Blood pressure was monitored using radio telemetry.

Results: Hemichannels and GJs composed of the Cx40 T152A mutant lacked both chemical and electrical conductance in vitro, and impaired the function of wild-type Cx40, Cx43 and Cx45, but not Cx37. Expression of Cx40T152A was restricted to the vascular endothelium in Cx40T152ATg mice and did not affect expression of endothelial Cx37. Endothelial expression of Cx40 T152A impaired endothelial, but not myoendothelial, GJ coupling. Cx40T152ATg mice displayed a significantly higher activity-dependent elevation in blood pressure relative to wild-type mice (WT: 111.3±2.5 mmHg, \(n=7\); Cx40T152ATg: 119.5±1.8 mmHg, \(n=7\); \(P<0.05\)) and a significantly increased relationship between heart rate and activity. In the absence of activity there was no difference in blood pressure between genotypes (WT: 103.6±2.0 mmHg, \(n=7\); Cx40T152ATg: 108.1±2.8 mmHg, \(n=7\)).

Conclusion: These data show that vascular endothelial Cx40 plays an important role in the regulation of blood pressure during activity, suggesting that functional impairment of endothelial Cx40 may lead to exercise-induced hypertension.
HBP 27: HYPERTENSION IN ZIMBABWE: A META-ANALYSIS TO QUANTIFY ITS BURDEN

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Background: Hypertension is a recognized global public health problem. Due to a lack of comprehensive national health data, the true magnitude of the problem in Zimbabwe is unknown, and underlying risk factors are not completely understood. Estimating the prevalence of hypertension in Zimbabwe is an important step to informing the development of effective prevention and control strategies.

Aim: To estimate the prevalence of hypertension in Zimbabwe and describe its trend since independence in 1980 using secondary source data.

Methods: We performed a comprehensive literature search of MEDLINE, EMBASE and Scopus databases, supplemented by an exploration of bibliographies cited in the articles identified so as to provide further studies. The search was restricted to community or population based studies that used either the WHO (BP ≥160/95 mmHg) or the 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (BP ≥ 140/90 mmHg) criteria to estimate the prevalence of hypertension in Zimbabwe. The pooled prevalence was calculated using random-effect modeling for meta-analysis. Heterogeneity was assessed using I2 statistics.

Results: After applying the inclusion and exclusion criteria, a total of 4 studies enrolling 4,829 participants between 1997 and 2010 across 5 provinces in Zimbabwe were identified and included in our review. The pooled prevalence of hypertension in the population was estimated to be 30% (95% CI 19–42%). The prevalence of hypertension increased over the 14-year study period (P<0.005), and appeared to be rising more rapidly in urban settings compared with rural settings.

Conclusion: Results from our meta-analysis, of an observed trend towards increasing hypertension prevalence in Zimbabwe, particularly in urban areas, is congruent with other studies documenting increasing hypertension prevalence in less developed countries. Hypertension management in Zimbabwe faces other challenges: economic, environmental and a high burden of communicable disease, particularly HIV. Further research is required to understand the reasons behind the increase in prevalence. This will help inform both its management and future prevention. There is an urgent need to develop policies to prevent, detect, treat and manage hypertension effectively in Zimbabwe.

HBP 28: PRAGMATIC METHOD TO ASSESS TREATED BLOOD PRESSURE FROM HOME BLOOD PRESSURE DIARIES: OPTIMAL STUDY

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Background: Home blood pressure (HBP) is a better predictor of end-organ damage, cardiovascular events and mortality than clinical measures. HBP is collected and interpreted in general practice in an ad hoc manner. One barrier to using HBP in general practice is the need to calculate average BP.

Aim: To develop a timely pragmatic method to assess BP control from patient diaries.

Methods: Seven-day HBP and 24-hour ambulatory BP (24-ABP) were measured on 286 patients with uncomplicated treated hypertension from 3 Australian centres. We sought the optimal number of HBP readings above threshold (≥135 mmHg) from the last 10 recorded that would best predict elevated 24-ABP SBP (≥130 mmHg). Sensitivity was tested by association with markers of end-organ damage.

Results: Participants were aged 64±8 years, 53% female, daily defined dose antihypertensive medications 2.4±1.4, clinic BP 127/75±14/10 mmHg. Having ≥3 of the last 10 systolic HBP readings ≥135 mmHg provided the best prediction of 24-ABP SBP (AUC=0.71). These individuals also had greater evidence of end-organ damage compared with those who did not meet these criteria.

Conclusions: BP is the most common condition managed in general practice and yet its management is less than ideal. It is usually managed using a clinic derived datum point rather than a community-based dataset that is more representative of true BP. To facilitate uptake of HBP monitoring it is proposed that GPs can simply collect and determine a summary statistic by the OPTIMAL method.

HBP 29: A SURGICAL MODEL OF HYPERTENSION AND LEFT VENTRICULAR HYPERTROPHY IN MICE BY SUPRARENAL AORTIC CONSTRICITION

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Background: Surgical models of hypertension and left ventricular hypertrophy (LVH) have been initially developed in larger species and later in the mouse to meet the demands of transgenic and gene inactivation studies in which mice remain the animal of choice. Surgery, however, is more technically challenging in mice. Suprarenal aortic constriction (SAC) is an approach to inducing high blood pressure as a result of renin-angiotensin-aldosterone-system activation, which is the target of many antihypertensive therapies.
Aim: To establish a murine model of SAC and, here, characterize SAC-induced hypertension and LVH.

Methods: SAC was performed in C57BL/6J mice at 8 weeks of age. The suprarenal aorta (just above both renal arteries) was tied on to a 29 gauge needle with a 7.0 silk suture and the needle was then removed. The diameter of the aorta was estimated to be reduced by approximately one-third of its original diameter. One week post-SAC, micromanometry and echocardiography were performed to determine cardiac function and structure, and tissues collected for analysis.

Results: Post-SAC, systolic blood pressure increased by 45±3 mmHg (sham 109±2 mmHg, n=19 vs. SAC 154±3 mmHg, n=17; P<0.0001), confirming induction of hypertension. Successful banding, defined as a systolic blood pressure >2 S.D. above the mean sham value, was achieved in 80%. LVH was evident from an increase in LV mass, enlargement of cardiomyocytes and, consistent with pathological hypertrophy, increased expression of ANP, BNP, β-MHC and α-SKA. Echocardiography showed thickening of the LV wall (h) and reduced LV chamber radius (R), resulting in an increase in the h/R ratio, which also indicates concentric hypertrophy development. This was accompanied by a reduction in cardiac output (−14%; n=9–11; P<0.05), ejection fraction (−8%; n=9–11; P<0.05) and fractional shortening (−11%; n=9–11; P<0.05). Furthermore, one week post-SAC there was evidence of LV remodelling with increased perivascular fibrosis.

Conclusion: SAC-induced hypertension and LVH in mice mimics the phenotype of hypertensive patients with pathological LVH. This surgical model is therefore a valuable research tool for understanding the molecular and biochemical changes underpinning LVH and for evaluating potential therapeutics in wild-type and genetically-modified mice.

HBP 30: REINSTATEMENT OF RESPIRATORY SINUS ARYTHMIA INCREASES STROKE VOLUME WITHOUT CONCOMITANT INCREASE IN OXYGEN DEBT IN RATS WITH LEFT VENTRICULAR DYSFUNCTION

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Background: Respiratory sinus arrhythmia (RSA) is the physiological phenomenon whereby heart rate is modulated by respiration. Whilst its physiological function is unknown, our mathematical modelling predicts that RSA saves cardiac energy. Cardiovascular diseases, including heart failure, are associated with a loss of RSA.

Aim: To test whether reinstating RSA would improve cardiac function post myocardial infarction (MI) using a novel central pattern generator (CPG) that couples heart rate to respiration.

Methods: Experiments were conducted on Wistar rats (male, 250–300 g) 3 days after left anterior coronary artery ligation (n=7). Rats were anaesthetized with isoflurane (1.2–1.8% in oxygen). The input signal to the CPG was diaphragmatic EMG. The CPG generated output stimulus was delivered to the right cervical vagus nerve. In cohort 1, arterial pressures, heart rate, respiratory rate, expired CO2, body temperature and instantaneous blood flow from the ascending aorta (i.e., stroke volume) were monitored. A generated output stimulus was delivered to the right cervical vagus nerve. In cohort 1, arterial pressures, heart rate, respiratory rate, expired CO2, body temperature and instantaneous blood flow from the ascending aorta were simultaneously and the effect of induced RSA versus tonic bradycardia on cardiac output was tested. In cohort 2, rats were subjected to RSA, tonic vagal nerve stimulation (VNS) or time-matched controls (n=8–10 per group) for 45 min and hearts were freeze-clamp collected and lactate concentration measured for changes in cardiac metabolic function. One-way ANOVA between treatments and post-hoc paired t-tests were performed. Data are presented as mean±SEM and P<0.05 was taken as significant.

Results: Cardiac dysfunction was verified by infarct size (43±7% of left ventricle) and elevated end-diastolic pressure (16±1 mmHg). Using the CPG, RSA of 15–76 beats per minute (bpm) bradycardia caused a 12±3% increase in stroke volume. RSA amplitude was not correlated with a greater increase in stroke volume (r²=0.00). Tonic VNS at matched average heart rate to RSA increased stroke volume by 8±1% (P=0.25 compared to RSA). Despite the increase in stroke volume, no difference in cardiac tissue lactate concentration was observed between RSA, tonic VNS and time-matched control post-MI rats (P=0.35).

Conclusions: Using a novel CPG device, we have demonstrated that, in an acute setting, reinstating RSA improves cardiac function. This may provide a novel therapeutic method of increasing cardiac output without generating an oxygen debt, at least in the rat. Ongoing studies are testing whether cardiac output is further optimized in a large pre-clinical animal model that has a heart rate comparable to that of humans.

HBP 31: IN VITRO CULTURE WITHOUT HUMAN SERUM OR WITH HUMAN SERUM AND METHYL DONOR ALTERS CARDIOMYOCYTE ENDOWMENT IN POSTNATAL LIFE


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Background: The cardiovascular system is vulnerable to perturbations during pregnancy. Previous studies have shown that an altered intrauterine environment can impact on cardiomyocyte endowment at birth and in early life.

Aim: To investigate whether in vitro culture and transfer of the embryo, which are important steps in assisted reproductive technologies and perturbations to the nutritional environment during the periconceptional period, alter cardiomyocyte endowment in postnatal life.

Methods: Embryos were either transferred to an intermediate ewe (ET) or cultured in vitro in the absence (IVC) or presence of human serum (IVCHS) and a methyl donor (IVCHS+M) for 6 days. Naturally mated (NM) ewes acted as controls. At 24 weeks, hearts were collected and slices were sampled using the smooth fractionator technique.
The estimation of cardiomyocyte number in the left ventricle was performed using design-unbiased stereological techniques. **Results:** IVC and IVCHS+M groups had an increased number of cardiomyocytes in the left ventricle compared to the IVCHS group, but neither of these groups differed from the NM and ET groups. This effect of treatment was present in only the male (P<0.05) and not the female lambs. **Conclusions:** The findings from this study highlight the differential effect of the composition of medium used for embryo culture on cardiomyocyte number in postnatal life. This can have implications for heart health in later life. The increased cardiomyocyte endowment could be due to alterations in processes involved in cardiac remodelling such as apoptosis and autophagy and this requires further investigation.

**HBP 32: IMPACT OF IN VITRO EMBRYO CULTURE AND TRANSFER ON BAROREFLEX SENSITIVITY IN EARLY POSTNATAL LIFE**


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**Background:** Previous studies have highlighted the ability of the nutritional environment of the oocyte and early embryo during the periconceptional period to alter blood pressure regulation in fetal and postnatal life. **Aim:** To investigate whether in vitro embryo culture and transfer, as well as manipulations to the nutritional environment during the periconceptional period, result in dysregulation of baroreflex control of blood pressure. **Methods:** Embryos were either transferred to an intermediate ewe (ET) or cultured in vitro in the absence (IVC) or the presence of human serum (IVCHS) and a methyl donor (IVCHS+M) for 6 days (d). Controls were naturally mated (NM) ewes. Mean arterial pressure (MAP) and heart rate (HR) were measured via an indwelling carotid artery cannula under basal conditions and during phenylephrine infusion. The relationship between MAP and HR was analysed using the logistic function and the maximal gain was obtained to compare the slopes between different treatment groups. One-way ANOVA was used to analyse the slopes from each curve (SPSS 18 for Windows, Statistical Package for Social Scientists Inc., IL, USA). **Results:** Basal MAP and HR were unchanged between the treatment groups. There was no significant difference between the upper plateau (P=0.059), and maximal gain coefficient obtained between the treatment groups (P= 0.50) and MAP50 (the pressure at the midrange of the curve) when compared to the NM (P=0.11). However, the males had a higher MAP50 than the females (P<0.05). **Conclusions:** The results suggest that embryo transfer and in vitro embryo culture do not alter basal MAP, HR or baroreflex sensitivity.

**HBP 33: HIGH BLOOD PRESSURE AS A DRIVER OF STRUCTURAL AND BIOMECHANICAL CHANGES IN MESENTERIC RESISTANCE ARTERIES IN CHRONIC KIDNEY DISEASE**

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**Background:** Pathological alterations in resistance artery structure and biomechanics are seen commonly in association with hypertension. **Aim:** To use pressure myography and histology to investigate mesenteric resistance artery structural stiffness and morphology in a genetic hypertensive rat model of chronic kidney disease (CKD). **Methods:** Pressure myography and histology were used to investigate mesenteric resistance artery structural stiffness and morphology in the Lewis polycystic kidney (LPK) rat, at early (12 weeks; n=7) and established (18 weeks; n=7) CKD time-points, relative to age-matched Lewis control rats (n=7 each). Rats were phenotyped for systolic blood pressure (SBP) and urine morphology in the Lewis polycystic kidney (LPK) rat, at early (12 weeks; n=7) and established (18 weeks; n=7) CKD time-points, relative to age-matched Lewis control rats (n=7 each). **Results:** Twelve and 18 week-old LPK exhibited eutrophic and hypertrophic inward remodelling, characterized by increased medial smooth muscle thickness, decreased lumen diameter, and unchanged and increased media cross-sectional area (MCSA) in 12 and 18 week-old LPK, respectively, relative to age-matched Lewis controls. Structural changes were not associated with hyperplasia, as no age or strain-related changes in nuclear density or number were found. Larger elastic-modulus/stress slopes (EM/σ: 24±2 vs. 6±0.3 and 5±1, respectively [P<0.001]; and collagen/elastin ratio: 8±2 vs. 2±0.5 and 4±1, respectively [P<0.05]). Regression analysis revealed SBP as a main predictor of wall/lumen ratio (r2=0.49; P=0.0003). **Conclusions:** These results indicate that mesenteric resistance arteries in the LPK model of CKD undergo vascular remodelling and stiffness. Hypertension likely predisposes to structural alterations in CKD.
**HBP 34: EFFECT OF BLOOD COLLECTION ON BLOOD PRESSURE: IMPLICATIONS FOR ASSESSING CARDIOVASCULAR RISK IN CLINICAL PRACTICE**

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**Background:** Doctors may conduct cardiovascular risk assessments that include blood collection and measurement of blood pressure (BP) within the same consultation period. The accuracy of risk assessment could be compromised if blood collection changes BP significantly, but this has not been investigated.

**Aim:** To determine whether blood collection changes BP significantly.

**Methods:** Twenty-four participants (56±11 years, 15 male), including 8 patients with type 2 diabetes and 16 healthy nondiabetics, had BP measured (iEM GmbH, Mobil-o-graph) before (x3), during (x1) and after (x3) blood collection on the contralateral arm in the seated position as per guidelines. The Mobil-o-graph device also provided hemodynamic measures of cardiac output, total vascular resistance and aortic stiffness. On a separate visit, BP was measured in duplicate after 10 minutes rest in the absence of blood collection, as an indication of research-grade clinic BP.

**Results:** Systolic BP (SBP) measured before, during or after blood collection was not significantly different from research-grade SBP (P>0.05 for all, whether diabetic or nondiabetic). There was a trend towards reduced total vascular resistance immediately after blood collection (P=0.1). This was, however, unrelated to changes in BP (r<0.21, P>0.33 all). Research-grade SBP significantly correlated with SBP measured before, during and after blood collection (intraclass correlation coefficient r>0.83; P<0.001 all).

**Conclusions:** SBP measured during blood collection is representative of research-grade clinic SBP and does not affect assessment of cardiovascular risk.

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**HBP 35: FACTORS AFFECTING BLOOD PRESSURE AND RENAL HEALTH IN YOUNG INDIGENOUS PREGNANT WOMEN**


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**Background:** Indigenous Australians have a high mortality and morbidity from cardiovascular disease and chronic kidney disease.

**Aim:** To study the health of young Indigenous pregnant women who have participated in the Gomeroi/Gaaynggal Artshealth program.

**Methods:** Women were aged between 14 and 41 years. Nine, 31 and 60% of the samples were collected in the first, second and third trimesters, respectively and samples were taken from 55, 33 and 11% of women on 1, 2 or 3 occasions. Further data relating to blood pressure was collected from antenatal records.

**Results:** Only 5 women ever recorded a systolic blood pressure ≥140 mmHg, and 6 women a diastolic pressure ≥90 mmHg. On the other hand, 18 women had urinary protein to creatinine ratios ≥30 mg/mmol, 6 had more than one record of proteinuria. Diastolic pressure and plasma cystatin C showed gestation dependent increases (each P<0.001). IgG antibody levels and C reactive protein (CRP) both showed a negative correlation with gestation age (P≤0.05). There were positive correlations between systolic pressure and CRP (rho=0.212, 106; P=0.029) and white blood cell (wbc) count (rho=0.225, 112; P=0.017). The correlations between diastolic pressure and these variables did not reach statistical significance, although there was a negative correlation between cotinine levels (a measure of exposure to cigarette smoke) and diastolic pressure (rho=−0.253, 83; P=0.02). Cotinine levels were also directly related to IgG levels (rho=−0.22, 99; P=0.026) and wbc count (rho=−0.279, 108; P=0.003). In women with a urinary protein/creatinine ≥30 mg/mmol, blood pressures, components of the circulating renin-angiotensin system, and inflammatory markers were similar to those measured in women without proteinuria. However, their albumin/creatinine levels were higher (P=0.001), as were their urinary angiotensinogen, ACE, prorenin and active renin/creatinine ratios (P= 0.012, 0.002, 0.021 and 0.002, respectively).

**Conclusions:** In this population of Indigenous Australian women, the incidence of high blood pressure is low, but there is an increased prevalence of proteinuria. Systolic blood pressure appears to be affected by the presence of inflammation, as evidenced by the relationships between systolic pressure, CRP and wbc count. The higher urinary angiotensinogen:creatinine ratio in pregnant women with proteinuria is in accordance with observations made in non-pregnant populations. As well the activity of all components of the intrarenal RAS that were measured was increased in these women, indicative of previously undetected severe renal dysfunction.
HBP 36: UREMIC TOXINS CONTRIBUTE TO CARDIAC FIBROSIS AFTER MYOCARDIAL INFARCTION: ROLE OF microRNAs

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Background: A decline in renal function is a common consequence of myocardial infarction (MI) resulting in increased cardiovascular events, known as cardiorenal syndrome (CRS). Although molecular mechanisms contributing to CRS are not well understood, a role for elevated plasma levels of the uremic toxin indoxyl sulphate (IS) and increased fibrosis have been described. MicroRNAs are small endogenously transcribed regulatory RNAs that modulate gene expression and regulate many cardiac processes involved in cardiac dysfunction.

Aim: To investigate, using a rat model, (i) whether MI leads to changes in expression of cardiac microRNA-21 and microRNA-29, both known to contribute to fibrosis and (ii) the effect of lowering plasma uremic toxins on cardiac expression of these microRNAs.

Methods: MI was induced by coronary artery ligation in male Sprague-Dawley rats. At 16 weeks cardiac function was measured prior to sacrifice. Cardiac tissues were assessed for molecular changes using quantitative real-time PCR, western blot analysis and histological methods. Fibrosis was evaluated by calculating the proportional area of picrosirius red stained matrix using image analysis. Cardiac microRNA-21 and 29b were measured from subtotal nephrectomy rats (STNx) to confirm the role of IS and renal damage. The effect of direct exposure of IS on cultured cardiac fibroblast cells was evaluated.

Results: The percentage area of cardiac fibrosis and delta serum IS levels were significantly higher in the vehicle MI group compared to sham animals (P<0.05 for both). Both were significantly attenuated by oral administration of AST-120, which absorbs toxins in the gastrointestinal tract (P<0.05 for fibrosis and P<0.001 for delta serum IS, compared to MI+Vehicle). MI significantly increased cardiac microRNA-21, collagen1A1, fibronectin-1 and TGFβ1 mRNA levels, as well as cardiac fibrosis and collagen 1 protein levels. Conversely, microRNA-29 expression was reduced in the heart. Treatment with AST-120 significantly reversed all of these changes. Compared to sham rats, level of cardiac microRNA-21 was increased (P<0.05) and microRNA-29b was decreased (P<0.01) in vehicle treated rats with STNx surgery. This change in microRNA levels was significantly attenuated by AST-120 treatment. Direct exposure to IS was sufficient to increase level of microRNA-21 and reduce level of microRNA-29b in cultured fibroblast cells.

Conclusions: We report a link between the beneficial effects of lowering circulating uremic toxins and microRNAs changes in the heart. Targeting microRNAs may provide a therapeutic target for the treatment of CRS.

HBP 37: TARGETING INFLAMMATION IN THE BRAIN DURING ANGIOTENSIN II-INDUCED HYPERTENSION

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Background: Hypertension is associated with oxidative stress and inflammation, and is a major risk factor for stroke.

Aims: To characterize inflammatory cell types in the brain during angiotensin II (Ang II)-induced hypertension and to test whether deletion of Nox2, a key mediator of inflammation in the brain, attenuates this inflammation.

Methods: Ang II was induced by coronary artery ligation in male Sprague-Dawley rats. At 16 weeks cardiac function was measured prior to sacrifice. Cardiac tissues were assessed for molecular changes using quantitative real-time PCR, western blot analysis and histological methods.

Results: In wild-type mice treated with Ang II, SBP was elevated (150±4 mmHg, n=20) compared to vehicle-treated mice (118±4 mmHg, n=17; P<0.0001). Similarly, Ang II increased SBP in Nox2-deficient mice (157±7 mmHg, n=11) compared with vehicle treatment (113±7 mmHg, n=9; P<0.005). Flow cytometric analysis of brain cell suspensions indicated an increase of microglia (CD45hiCD11b-F4/80-; 2.1-fold), total leukocytes (CD45hi; 3.9-fold) and leukocyte subsets including total monocytes (CD45hiLy6C-; 2.2-fold), neutrophils (CD45hiLy6G-; 3.9-fold) and T cells (CD45hiCD11b-CD3+; 3.0-fold) in Ang II-infused wild-type mice compared to vehicle (n=12–19; all P<0.05). In contrast, there was no difference in any inflammatory cell numbers between Nox2-deficient mice treated with Ang II or vehicle (n=9–11; P>0.05). Quantitative PCR analysis indicated no effect of Ang II on mRNA expression of interleukin-6, chemokine (C-C motif) receptor 2, and interleukin-10, in the brains of wild-type mice compared to vehicle treatment (n=8, all P>0.05).

Conclusions: Ang II induces hypertension that is associated with an increase in several inflammatory cell types in the brain. However, the inflammatory cell infiltration of the brain, but not the hypertension, is dependent on Nox2 oxidase expression.

HBP 38: PERFORMANCE OF THE BPTRU AUTOMATIC BLOOD PRESSURE MONITOR IN THE ASSESSMENT OF BLOOD PRESSURE CONTROL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background: Optimal blood pressure (BP) control is essential in the management of chronic kidney disease (CKD).
Although automated BP monitoring (ABPM) provides accurate BP assessment, it may not be readily available. Hence, it is important to identify a simple and accurate BP monitoring device that can be used in the clinic setting. **Aim:** To compare the accuracy of the BpTRU device with 24-hour ABPM in diagnosing and monitoring of hypertension in patients with CKD.

**Methods:** Casual clinic BP was measured in 136 CKD patients using the BpTRU device and a mercury sphygmomanometer. These measurements were followed by 24-hour ABPM. Using the mean 24-hour ABPM as reference standard, the proportion of patients with white coat hypertension (WCH) and masked hypertension (MHT) were determined for readings obtained using the BpTRU and mercury devices. Agreement between the methods was examined by Bland-Altman plots with ABPM as the gold standard. BP data were reported as median with interquartile range.

**Results:** The median mercury, BpTRU and 24-hour average ambulatory BP readings were 134 (124–148)/74 (67–81), 127 (115–137)/72 (65–79) and 133 (124–142)/72 (66–78) mmHg, respectively. Systolic BP (SBP) obtained using the BpTRU device was significantly lower than the 24-hour average ambulatory SBP (P<0.005). In contrast, SBP obtained using the mercury sphygmomanometer was higher than the 24-hour average ambulatory SBP (P<0.005). Casual clinic BP measurement using the BpTRU device demonstrated WCH in 1.5%, MT in 43%, controlled HT in 32% and sustained HT in 23%. Using a mercury sphygmomanometer, WCH was seen in 2%, MHT in 31%, controlled HT in 30% and sustained HT in 37%. Bland-Altman plot showed good agreement between these BP measurement techniques.

**Conclusion:** In the clinic setting, BP control in the CKD group was better determined by the mercury sphygmomanometer than the BpTRU monitor. The BpTRU device underestimated SBP and misclassified more cases of true HT as masked HT than a mercury sphygmomanometer.

**HBP 39: MORBIDITY, HOSPITALIZATION AND MORTALITY FROM HEART FAILURE IN AUSTRALIA: A SYSTEMATIC REVIEW**

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**Background:** In the absence national population-based evidence on heart failure,; synthesis of available data would be important to describe the burden of heart failure in Australia.

**Aim:** To systematically review the literature on morbidity, mortality and hospitalization from heart failure in Australia.

**Methods:** Keywords were used to search the literature in Embase, MEDLINE and Scopus. Non-indexed relevant government publications were also included. Findings extracted from articles included in the review were synthesized according to objectives of the review.

**Results:** Of the 493 articles screened, 19 studies were included. Incidence of heart failure in those aged 60 years and over was estimated as 0.6%, and a crude incidence rate of 2.4% was noted in elderly subjects with prior diagnoses of hypertension. The prevalence of heart failure for the general population, weighted from these studies, was approximately 1.6% and 5.5% in those aged 60 years and over. Similarly, the weighted incidence rate of hospitalization was 0.53% for the general population and was 80–89% for individuals aged 65 years and over. According to studies that reported mortality data, heart failure is an underlying cause of death for 2.0–3.2% of total deaths in Australia.

**Conclusions:** The results show that the burden of heart failure, especially in elderly Australians is high. Most studies were based on patient records and extrapolation of such data. Thus, little is known about the population-based burden of heart failure, as well as its epidemiology after the onset of other cardiovascular diseases.

**HBP 40: PRESSURE-INDUCED T CELL HOMING IN THE AORTA**

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**Background:** T cell infiltration into target cardiovascular organs, such as the aorta and kidney, is thought to play a role in the etiology of hypertension and disease progression and to promote organ damage. The mechanisms underlying T cell recruitment and infiltration are not known. As we have shown previously high intraluminal pressure can induce endothelial cell activation and cytokine release. We hypothesize that factors released from endothelial cells under pressure can promote T cell recruitment and infiltration.

**Aim:** To examine the contribution of pressure-induced endothelial cell activation to T cell recruitment.

**Methods:** T cell migration was assessed in the presence of supernatants collected from human umbilical endothelial cells (HUVECs) that were untreated, treated with TNFα for 24 hours (10 ng/mL), pressurized at 120 mmHg for 24 hours or medium alone with the known migration stimulus, CCL21 (100 ng/mL). The number and phenotype of migrated T cells was assessed via antibody-directed flow cytometric analysis. In addition, we examined whether T cell migration was dependent on factors released by the perivascular adipose tissue (PVAT) surrounding the aorta. PVAT was harvested from the thoracic aorta of 14 week-old normotensive WKY and hypertensive stroke-prone spontaneously hypertensive (SHR-SP) rats and cultured for 24 hours.

**Results:** The number of migrated T cells in the presence of supernatants collected from untreated cells (773±52, n≥6) was lower than supernatants collected from pressurized cells (8491±2877, n≥2; P<0.01), TNFα-treated cells (36621±1354, n≥2; P>0.001) and CCL21 treated medium (23961±1538, n≥2; P<0.01), with approximately 48% of migrated T cells in all treatment groups comprising the inflammatory T helper 1 subtype. The migrated T cells in the presence of...
**HBP 41: MATERNAL HYPOMAGNESEMIA DOES NOT CAUSE PROGRAMMED CHANGES TO CARDIOVASCULAR PHYSIOLOGY IN ADULT OFFSPRING**

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**Background:** Magnesium (Mg) is essential for development and the maintenance of normal cellular processes. Clinical and experimental evidence suggests a strong inverse relationship between Mg deficiency and the development of cardiovascular disease. It is also well established that maternal perturbations such as dietary nutrient deficiencies can result in an increased susceptibility to disease in offspring, including high blood pressure. However, few studies have explored the implications of maternal Mg deficiency and programmed cardiovascular outcomes in offspring.

**Aim:** To establish a model of Mg deficiency prior to and during pregnancy using CD1 female mice and investigate cardiovascular outcomes in adult offspring at 6 months of age.

**Methods:** Female CD1 mice (7 weeks old) received either a control Mg diet (0.2% w/w Mg), or Mg deficient diet (MMD) (0.02% w/w). Mice were maintained on this diet for 4 weeks prior to mating and throughout gestation. After 4 weeks on the diet, mice were mated overnight with age-matched CD1 males. Mice were allowed to litter naturally and offspring were reared to age 6 months for analyses. Offspring were implanted with radiotelemetry probes to measure blood pressure. Stress tests were conducted following probe implantation to measure cardiovascular reactivity (changes in mean arterial pressure and heart rate during stress). These included a physical stressor (restraint stress), a non-physical stressor (dirty cage swap), and a “positive stressor” (almond feeding test).

**Results:** Offspring were born growth restricted, but underwent catch-up growth after weaning. We found no change in basal mean arterial pressure or heart rate in adult offspring. Tests for cardiovascular reactivity following restraint, cage swap and feeding tests revealed no change in mean arterial pressure or heart rate between groups or sexes.

**Conclusion:** Despite evidence suggesting that poor maternal nutrition and fetal growth restriction can program hypertension in adult offspring, our findings demonstrate that maternal Mg deficiency does not cause programmed changes in basal cardiovascular physiology or cardiovascular reactivity to stress in adult offspring.

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**HBP 42: ARTERIAL IMPEDANCEMismatching FAILS TO EXPLAIN CENTRAL PRESSURE AUGMENTATION: AORTIC RESERVOIR FUNCTION MAY BE THE PREVAILING FACTOR**

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**Background:** Central augmentation pressure (AP) and augmentation index (AIx) are believed to occur due to wave-reflection from central-to-peripheral arterial impedance mismatching. High-impedance from stiffened peripheral vasculature should cause increased wave reflection, AP and AIx. Recent evidence suggests, however, that aortic reservoir function may be a more dominant contributor to AP and AIx.

**Aims:** To determine (i) the relationship of central-to-peripheral arterial impedance mismatch with AP and AIx, and (ii) independent correlates of central BP augmentation.

**Methods:** Carotid-to-femoral (aortic) pulse wave velocity (aPWV) and carotid-to-brachial PWV (bPWV) were measured in 359 individuals (aged 61±9, 49% male). Central AP, AIx and aortic reservoir pressure were derived from radial tonometry. Participants were stratified according to high- (bPWV > aPWV, n=118) or low-impedance mismatch (bPWV ≤ aPWV, n=241).

**Results:** Central AP and AIx were significantly higher in participants with low-impedance mismatch compared to those with high-impedance mismatch (11±6 vs. 8±6 mmHg; P=0.001 and 24±11 vs. 21±13 %; P=0.01). Impedance mismatch (bPWV–aPWV) was weakly and negatively associated with AP (r=–0.17; P=0.001), and was not associated with AIx (r=–0.05; P=0.31). Conversely, aortic reservoir pressure was a strong correlate of AP (r=0.77; P<0.001) and AIx (r=0.58; P<0.001). In multivariable linear regression, reservoir pressure predicted AP and AIx independent of age, sex, systemic vascular resistance, heart rate, mean arterial pressure and height (standardized β=0.45 and 0.19; P<0.001 for both, respectively).

**Conclusions:** Conflicting with conventional theoretical expectations, high-impedance mismatch between central and peripheral arteries does not cause higher AP or AIx. Aortic reservoir function, rather than discrete wave-reflection from arterial impedance mismatching may better explain AP and AIx.
**Background:** Heart failure with preserved ejection fraction (HFPEF) accounts for ≈50% of heart failure cases. Patients with HFPEF do not respond to current heart failure treatment therapies and the mechanisms leading to HFPEF are unknown. Critically, HFPEF patients display increased cardiac fibrosis compared to non-HFPEF patients. Endothelial-to-mesenchymal transition (EndoMT) is a major contributor to the development of cardiac fibrosis, but its initiating factors remain unknown. One major risk factor implicated in the development of HFPEF is hypertension, which has also been shown to promote endothelial activation and vascular inflammation, both of which are observed in HFPEF patients and are essential for EndoMT. We hypothesize that high blood pressure per se promotes EndoMT.

**Aim:** To examine whether high intraluminal pressure contributes to EndoMT-induced fibrosis.

**Methods:** We examined endothelial to fibroblast transition in human umbilical venous endothelial cells (HUVECs) after 1, 3, and 5 days of treatment. mRNA expression of fibroblast and endothelial genes was assessed by real-time PCR in HUVECs untreated or treated with TGFβ1 (10 ng/mL), TGFβ2 (10 ng/mL) or pressurized to 120 mmHg. We observed consistently greater expression of the fibroblast genes vimentin and alpha-smooth muscle actin (αSMA), in the TGFβ1 and 2 treated HUVECs compared to the untreated cells, particularly at 3 days when TGFβ-treated cells expressed significantly greater vimentin mRNA levels compared to untreated cells (>2 fold change). Concurrently, expression of the endothelial gene, CD31, was consistently reduced in the TGFβ-treated cells compared to the control group (>2 fold change) suggesting that these cells were undergoing transition away from an endothelial and towards a fibroblast phenotype. HUVECs exposed to 120 mmHg over 1, 3, and 5 days elicited a consistently lower CD31 gene expression compared to unpressurized cells. Conversely, both vimentin and αSMA mRNA increased in the pressurized cells relative to the unpressurized cells at 5 days (≈3 fold change in vimentin; >2 fold change in αSMA), suggesting that exposure to increased pressure promotes transition of endothelial cells towards a fibroblast phenotype.

**Conclusions:** High intraluminal pressure per se induces EndoMT, observed by a reduction in endothelial expression profiles and an increase in a mesenchymal/fibroblast phenotype. The study suggests that hypertension-induced EndoMT may contribute to cardiac fibrosis and may provide a novel therapeutic target to prevent development of fibrosis in HFPEF.

**HBP 45: THE EFFECTS OF POSITIVE ALLOSTERIC MODULATION OF GABAA RECEPTORS ON STRESS AND HYPERTENSION IN SCHLAGER HYPERVENTILATIVE MICE**

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**Background:** An exaggerated pressor response to stress has been linked to the subsequent development of hypertension. Hypertensive Schlager mice (BPH/2J) have neurogenic hypertension associated with abnormal reactivity of neurons within the forebrain integrating the response to aversive stress. Recent studies suggest they also have functional and molecular differences in GABAA receptors compared with their normotensive counterparts. Allopregnanolone is an endogenous neurosteroid reduced by chronic stress and when administered, decreases anxiety by positive allosteric modulation of GABAA receptors.

**Aims:** To determine if allopregnanolone reduces the pressor effects of stress and basal mean arterial pressure (MAP) in BPH/2J mice.

**Methods:** Male BPN/3J (n=9) and BPH/2J (n=5–7) mice received allopregnanolone (5 mg/kg/day) or its vehicle via subcutaneous minipumps for two weeks. Prior implantation of telemetric probes enabled recordings of MAP, heart rate (HR) and locomotor activity both prior to and following minipump implantation. The cardiovascular response to aversive (dirty cage switch and restraint) and non-aversive (feeding) stress tests, as well as ganglionic blockade with 5 mg/kg pentolinium were recorded before and following minipump implantation. Changes in levels of GABAA receptor mRNAs were assessed by quantitative RT-PCR.

**Results:** Allopregnanolone reduced systolic arterial pressure (−7.4 mmHg; *P<0.01) and diastolic arterial pressure (−4.5 mmHg; *P=0.02) and attenuated the depressor response to pentolinium in BPH/2J mice, whereas no effect on MAP was observed in BPN/3J mice. Allopregnanolone produced marked reductions in the pressor response to both cage switch and feeding stress (−20%; *P<0.01) in BPH/2J mice, whilst increasing the pressor response to aversive stress in BPN/3J mice (+33–48%; *P<0.001). In addition, allopregnanolone selectively increased the levels of mRNAs encoding α4 and δ subunits of GABAA receptor which mediate tonic inhibition in the hypothalamus of BPH/2J mice (1.7–4.8 fold; *P<0.05).

**Conclusions:** The selective antihypertensive and stress inhibitory effects of allopregnanolone in BPH/2J hypertensive mice suggests that allosteric modulation of GABAA receptors in the hypothalamus may be a major cause of hypertension in this model.
HBP 46: AUGMENTATION INDEX DIFFERS IN PACED SUBJECTS WITH DIFFERENT PACING MODALITIES, INDEPENDENT OF HEART RATE AND BLOOD PRESSURE

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**Background:** The influence of heart rate (HR) on augmentation index (Aix) is well established. Studies have also shown a strong correlation between left ventricular ejection duration (ED) and Aix. However, in patients with indwelling pacemakers, different pacing modalities may implicate different cardiac hemodynamics and thus possibly affect Aix.

**Aim:** To investigate the effect of pacing modality (PM) on ED, blood pressure (BP) and Aix.

**Methods:** Cuff-based pulse wave analysis was performed in 38 subjects (7 females, aged 80±7 SD years) with pacemaker implants at 5 HRs (60, 70, 80, 90 and 100 bpm) in a supine position. Brachial BP waveform was measured, and central BP, Aix and ED were determined from a derived central BP waveform. Data were analysed according to whether the subjects had atrial pacing (Ap), atrioventricular pacing (ApVp), or ventricular pacing (Vp) throughout the 5 pacing steps.

**Results:** When compared at the same HR using one-way ANOVA, PM did not affect central or brachial BP, nor pulse pressure (PP) except at 80 bpm, where PP was 44±2 mmHg for Ap, 40±2 mmHg for ApVp and 34±3 mmHg for Vp (mean±SEM; P=0.023). At all HRs, both Aix and ED were significantly lower in subjects with ApVp (representative data at 60, 80 and 100 bpm, respectively: Aix = 28±2%; 25±2% and 16±2%; ED = 335±5 ms, 302±5 ms and 277±4 ms) and Vp (Aix = 25±2%, 19±3% and 11±3%; ED = 313±5 ms, 294±8 ms and 260±5 ms) than those with Ap (Aix = 44±3%, 40±3% and 28±2%; P<0.001 at all 3 HRs; ED = 356±9 ms, 324±7 ms and 292±5 ms; P<0.001 at 60 and 100 bpm; P=0.02 at 80 bpm). In a multilevel linear mixed model, HR and PM were both shown to have main effects and significant interaction with ED on Aix (HR: X²(2) = 140.5; P<0.001; PM x ED: X²(2) = 21.0; P<0.001; HR x ED: X²(2) = 14.1; P=0.007; PM x ED: X²(2) = 18.5; P<0.001).

**Conclusions:** At constant HR, PM affects Aix, likely due to ED differences. Further investigation is required to determine other parameters that may influence Aix with different PMs. This can have implications on the current method of standardizing Aix measurements for HR only, in particular for paced patients.

HBP 47: REMOTE LIMB ISCHEMIC PRECONDITIONING: CONTRIBUTION OF VAGAL INNERVATION TO PROTECTION AGAINST MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

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**Background:** Coronary reperfusion is the primary treatment for a heart attack to reduce infarct size, but the reperfusion itself causes myocyte death. Myocardial reperfusion injury can be reduced by prior transient limb ischemia, which induces remote ischemic preconditioning (RIPC) that further reduces infarct size. The mechanisms by which RIPC causes cardioprotection are unclear, but are thought to include neural and humoral mechanisms.

**Aim:** To determine the role of vagal innervation in determining the cardioprotective effect of RIPC.

**Methods:** Anesthetised sheep were randomly allocated into 4 groups, (i) Sham conditioning (n=6), (ii) RIPC (n=6), (iii) Proximal vagal denervation + RIPC (n=6) and (iv) Distal vagal denervation + RIPC (n=6). RIPC involved 3×5 min occlusions of the iliac artery. Subsequently, the animals underwent 1 hour coronary ischemia followed by 3 hour reperfusion. Hemodynamic parameters were measured throughout the protocol. At the end of 3 hours of reperfusion the heart was excised and infarct size was measured.

**Results:** There were no clinically relevant significant differences in hemodynamic parameters among the 4 treatment groups. Compared with Sham animals, RIPC significantly reduced infarct size following IR (infarct size/area-at-risk, 82±5% vs. 49±7%, respectively; P<0.05). Distal vagotomy enhanced RIPC protection (31±7% infarct size) compared with proximal denervation (69±8% infarct size; P<0.05).

**Conclusions:** These data confirm the potential clinical benefits of limb RIPC against myocardial IR injury. Furthermore, they indicate that bilateral resection of the vagus nerves distal to the heart enhanced the cardioprotective effect of RIPC against reperfusion injury. This finding adds complexity to previous studies indicating that parasympathetic blockade with ganglion blockers inhibited the cardioprotective effect of RIPC against reperfusion injury.

HBP 48: ROLE OF THE ANGIOTENSIN TYPE 2 ON MACROPHAGE FUNCTION AND PHENOTYPE

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**Background:** The pro-inflammatory actions of macrophages are well known to contribute to cardiovascular disease. Macrophages also express components of the renin-angiotensin system, including the anti-inflammatory angiotensin type 2 (AT2) receptor (AT2R).

**Aim:** To investigate the role of direct AT2R stimulation on macrophage cytokine production and phenotype.

**Methods:** To induce cytokine production, RAW264.7 and bone marrow-derived macrophage (BMDM) cell lines were stimulated with lipopolysaccharide (LPS) (10 ng/mL) for 6 hours. Treatment groups included an unstimulated control, and LPS in the presence of vehicle, an AT2R peptide agonist, CGP42112 (0.1–10 μM), an AT2R nonpeptide agonist, compound 21 (C21; 0.1–10μM). Pro-inflammatory mediators (TNF-α, CCL2 and IL-6), regulatory cytokine IL-10
and M1/M2 polarization were measured in treated macrophages using flow cytometry. Quantitative analysis of cytokine secretion was performed using a cytometric bead array.

**Results:** The proportion of LPS-induced TNF-α, IL-6- and CCL2-producing BMDMs was significantly reduced by ~13% (n=14; P<0.05), ~23% (n=12; P<0.01) and 23% (n=7; P<0.05), respectively, when co-treated with peptide AT2R agonist, CGP42112. Consistent with these reductions, C21 also significantly reduced TNF-α (~20%; n=14; P<0.01), IL-6- (18%; n=12; P<0.01) and CCL2-production (~24%; n=7; P<0.05) in BMDMs. C21 was also effective at inhibiting the proportion of TNF-α (~15%; n=13; P<0.001) and CCL2-production (~27%; n=6; P<0.05) in RAW264.7 macrophages. LPS also increased IL-10-producing cells in both RAW264.7 (~38%; n=6; P<0.05) and BMDM (~50%; n=11; P<0.01), which appeared to be augmented with CGP42112 co-treatment (P<0.06).

**Conclusions:** Our results indicate that direct AT2R stimulation inhibits LPS-induced pro-inflammatory cytokine production, which may be associated with a modest increase in IL-10 production. Therefore, the AT2R represents a potential therapeutic target to control macrophage-dependent inflammation associated with cardiovascular disease.

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**HBP 49: VISUALIZATION OF INFILTRATING T CELLS AND THEIR DYNAMIC MOVEMENTS WITHIN AORTA FROM ANGIOTENSIN II-INFUSED MICE**

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**Background:** Hypertension is now known as an inflammatory disease and is associated with increased T cell infiltration into blood pressure-controlling organs. Classically, following activation, T cells migrate to sites of inflammation in the periphery and, when presented with cognate antigens, they undergo secondary activation, proliferation and mount a local immune response. Although T cells are well known to infiltrate the perivascular fat surrounding the aorta during experimental hypertension, whether they are activated in response to local antigen presentation remains unknown.

**Aims:** To develop a novel explanted aorta live-cell imaging assay to confirm whether hypertensive T cells infiltrate hypertensive vessels ex vivo and define the kinetics of their movement.

**Methods:** Splenic T lymphocytes were isolated from vehicle (normotensive) or angiotensin (Ang) II-infused (0.7 mg/kg/day) C57BL6/J mice using Miltenyi magnetic microbeads. T cells were subjected to 48 hour pre-stimulation with anti-CD3 and anti-CD28 antibodies. Stimulated T cells were then labelled with fluorescent labels CFSE (10 μM; Vehicle) or seminaphthorhodfluoro-1 (SNARF-1; Ang II), and then co-incubated simultaneously with explanted aorta with perivascular fat intact from separate normotensive or hypertensive mice. Following 16 hour incubation, 3D time-lapse video recordings were obtained to record dynamic T cell movements. Imaris software was used to count total T cell infiltrate, track cell velocity and determine the proportion of cells in motion.

**Results:** We observed marked infiltration of T cells isolated from hypertensive mice into hypertensive aorta compared to aorta from normotensive mice. These infiltrated were only localized to the perivascular fat of the aorta. However, minimal T cell movements and slower T cell velocity (μm/min) were observed within hypertensive aorta compared to in vehicle-treated aorta.

**Conclusion:** Our novel explanted aorta model confirmed the preference for T cell infiltration and/or recruitment into hypertensive aorta. Slowing of T cell velocity and a greater proportion of static T cells is suggestive of increased dynamic T cell interactions with antigen presenting cells, and recognition of cognate antigen locally within the vessel wall during hypertension.

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**HBP 50: A NOVEL SPONTANEOUSLY HYPERTENSIVE, DIET-INDUCED, ATHEROSCLEROSIS-PRONE MOUSE: A POTENTIAL NEW MODEL OF METABOLIC SYNDROME?**


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**Background:** Metabolic syndrome (MetS) is a prevalent multifactorial condition comprising glucose intolerance, insulin resistance, central obesity, dyslipidemia and hypertension and is associated with an elevated risk of both type 2 diabetes and cardiovascular disease (CVD). To date, research efforts to identify novel therapeutic treatment strategies to reduce the disease burden of MetS have been limited by the lack of an appropriate animal model that mimics the human condition.

**Aim:** To examine whether a novel spontaneously hypertensive atherosclerosis-prone mouse (BPHxApoe−/−) displays traits of the MetS.

**Methods:** Schlager high blood pressure (BPH) mice were crossed with apolipoprotein E knockout (Apoe−/−) mice to create BPHxApoe−/− mice (hypertensive, atherosclerotic-prone mouse: HAM). Eight week old Apoe−/− and HAM mice were fed either a western type diet (WTD; 21% fat, 1.2% cholesterol) or normal chow (NC; 4.8% fat, 0% cholesterol) for 16 weeks and underwent metabolic profiling throughout the treatment period.

**Results:** The hypertensive phenotype was confirmed in all HAM, with no effect of the diet. Total plasma cholesterol was comparable in the HAM and Apoe−/− mice, with both strains developing hypercholesterolemia when fed the WTD. Intriguingly, the weight gain in the BPHx mice fed the WTD comprised two distinct subgroups, which we stratified into standard <40 g (33.25±0.69 g) and overweight >40 g (43.6±0.76 g). An oral glucose tolerance test revealed that the overweight HAM had impaired glucose disposal and hyperinsulinemia, suggesting insulin resistance. Atherosclerotic lesion area was similar between WTD-fed HAM and Apoe−/− mice. There was, however, a tendency for a more unstable lesion, since we observed increased macrophage and lipid content in the plaques of the HAM.

**Conclusion:** A subset of HAM fed a WTD display dyslipidemia, elevated adiposity, hypertension, insulin resistance
Results:

We subsequently tested their cerebrovascular responsiveness (CVR) to cognitive stimuli by recording changes in MBFV from pre-pulsatility index (PI, a measure of arterial stiffness) were averaged over 30s in both left and right middle cerebral arteries (MCA).

Adults with non-insulin dependent T2DM and 13 controls after sitting for 10 min. Mean cerebral blood flow velocity (MBFV) and sensitivity and HbA1c) and basal MBFV, PI or cognitive performance. Reproducibility measures were excellent, e.g. ICC for PI were 0.987 (left) and 0.993 (right). Despite similar MMSE scores (mean score of 28.6 ± 1.4 for both groups), adults with T2DM tended to perform poorer in all cognitive tests, particularly in the N-back task (working memory). Importantly, CVR to the N-back task was lower in T2DM (P=0.057 [left]; P=0.011 [right], independent t-test); however, this deficit did not correlate with task performance.

Conclusion: With the limited data available, we are able to show for the first time that a cognitive deficit in T2DM is associated with an impaired ability to supply blood to the anterior brain region in response to the specific cognitive stimulus. This preliminary result suggests that cerebrovascular disease may contribute to deficits in working memory, executive function or processing speed in those with T2DM, with significant clinical implications for self-care health behaviours. We are evaluating more participants to gain a clearer understanding of these relationships.

HBP 51: DOES CONTRALATERAL SUPPRESSION AT ADRENAL VEIN SAMPLING PREDICT OUTCOME FOLLOWING UNILATERAL ADRENALECTOMY FOR PRIMARY ALDOSTERONISM? A RETROSPECTIVE STUDY

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Background: In primary aldosteronism (PA), adrenal vein sampling (AVS) is the most reliable method of distinguishing unilateral from bilateral disease. In AVS aldosterone/cortisol ratios (A/F) are used to correct aldosterone concentration for dilution from non-adrenal blood. Comparisons are then made between left, right and peripheral A/F ratios. Criteria for interpretation vary widely and there is no clear evidence of which method is most accurate. Most units use the laterisation index (LI); A/F dominant : A/F non-dominant with a cut-off value varying from ≥ 2 - 4 for unstimulated AVS to indicate unilateral disease. We have for many years used the criteria of ‘contralateral suppression’ (CS), defined as: A/F (adrenal) ≤ A/F (peripheral) on the unaffected side, combined with a ratio of ≥ 2 times peripheral on the affected side. Patients with one side clearly dominant but without CS are however sometimes offered surgery, depending on their particular characteristics and wishes. The importance of contralateral suppression in AVS interpretation is unclear, and we therefore performed a retrospective study to determine if CS in unilateral PA was associated with blood pressure (BP) and biochemical outcomes.

Methods: All patients who underwent unilateral adrenalectomy for PA at the Princess Alexandra Hospital between 2000 and 2014 were included for review if AVS was successful (based on gradients between adrenal and peripheral cortisol of ≥3 bilaterally), if the LI was ≥2 and if they had ≥6 months of post-operative follow up. Cases were reviewed for BP and biochemical outcomes with respect to the presence and degree of CS.

Results: 80 patients were available for review, and 66 had CS. Baseline characteristics were similar. At post-operative follow up, those with CS had lower systolic BP (SBP; 128mmHg vs. 143mmHg p=0.001), a greater proportion with cure or improvement of hypertension (95% vs. 64%, p=0.0034), a greater proportion with biochemical cure of PA on fluorocortisone suppression testing (43/49 [88%] vs. 4/9 [44%], p=0.002) and were on a lower median number of antihypertensive medications (0 vs. 1.5 p=0.0032).

In a multivariate linear model, the degree of CS and pre-operative SBP were both significantly correlated with post-operative SBP , but LI, gender and age were not.

Conclusions: In this retrospective study of the contribution of CS to the interpretation of AVS, the presence of CS correlated with good BP and biochemical outcomes from surgery. This suggests that CS should be a factor in deciding whether to offer surgery for treatment of PA.

HBP 52: A PILOT INVESTIGATION OF CEREBROVASCULAR RESPONSIVENESS TO A NEUROPSYCHOLOGICAL TEST BATTERY IN ADULTS WITH TYPE 2 DIABETES MELLITUS

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Aim: Our research examines the dependence of cognitive performance on cerebrovascular perfusion. Progressive arterial disease in type 2 diabetes mellitus (T2DM) may predispose individuals to greater risk of premature cognitive decline. We are conducting a pilot cross-sectional study in adults with T2DM and age- and gender-matched controls without T2DM to see whether limitations in the ability of cerebral vessels to supply blood in response to psychological stimuli predict poorer cognitive performance.

Method: Cognitive tests and transcranial Doppler (TCD) ultrasound assessments of cerebral blood flow were conducted in 31 adults with non-insulin dependent T2DM and 13 controls after sitting for 10 min. Mean cerebral blood flow velocity (MBFV) and pulsatility index (PI, a measure of arterial stiffness) were averaged over 30s in both left and right middle cerebral arteries (MCA). We subsequently tested their cerebrovascular responsiveness (CVR) to cognitive stimuli by recording changes in MBFV from pre-pulsatility index (PI, a measure of arterial stiffness). Reproducibility measures were excellent, e.g. ICC for PI were 0.987 (left) and 0.993 (right). Despite similar MMSE scores (mean score of 28.6 ± 1.4 for both groups), adults with T2DM tended to perform poorer in all cognitive tests, particularly in the N-back task (working memory). Importantly, CVR to the N-back task was lower in T2DM (P<0.057 [left]; P=0.011 [right], independent t-test); however, this deficit did not correlate with task performance.

Conclusion: With the limited data available, we are able to show for the first time that a cognitive deficit in T2DM is associated with an impaired ability to supply blood to the anterior brain region in response to the specific cognitive stimulus. This preliminary result suggests that cerebrovascular disease may contribute to deficits in working memory, executive function or processing speed in those with T2DM, with significant clinical implications for self-care health behaviours. We are evaluating more participants to gain a clearer understanding of these relationships.
HBP 53: DOES INCREASING MATERNAL INSULIN SENSITIVITY DURING THE PERICONCEPTIONAL PERIOD CORRECT THE CARDIAC DEFICITS INDUCED BY INTRAUTERINE GROWTH RESTRICTION IN THE SHEEP FETUS?

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Background: Intrauterine growth restricted (IUGR) fetuses have fewer cardiomyocytes and a susceptibility to cardiac hypertrophy and thus ischemic heart disease in adult life. Fetal adaptations to IUGR impact on the abundance of molecules that regulate cardiac growth and metabolism. Few studies have attempted to correct these cardiac deficits.

Aim: To intervene in pregnancies at a risk of IUGR by increasing maternal insulin sensitivity during the periconceptional period and investigate the expression of genes that regulate cardiac growth and development in the late gestation sheep fetus.

Methods: Non-pregnant ewes underwent a carunclectomy to induce placental restriction (PR) and IUGR. In these Control and PR ewes (factor 1: treatment), miniosmotic pumps were inserted subcutaneously one week before mating to deliver either vehicle or rosiglitazone (8 mg/day; factor 2: maternal periconceptional drug exposure) and removed 6 days after mating, resulting in 4 groups. Samples of the left ventricle were collected at 140 days gestation (term, 150 days) and specific mRNAs were measured by quantitative real-time PCR. Data were analysed using a 2-way ANOVA (factors: treatment and maternal periconceptional drug exposure) using SPSS.

Results: PR fetuses were smaller than Controls regardless of periconceptional exposure to vehicle or rosiglitazone. The levels of mRNAs encoding key molecules involved in the cell cycle (SPAG5, BIRC5, CDC2), glucose metabolism (PDK-4), fatty acid metabolism (PPAR-γ, PGC-1α, CPT-1, FABP5) and apoptosis (BCL-2) were increased in PR fetuses compared to Controls, while mRNA encoding the cell cycle inhibitor p21 was decreased, regardless of maternal periconceptional exposure to vehicle or rosiglitazone. Maternal rosiglitazone exposure during the periconceptional period increased mRNA encoding LC3β, a marker of autophagy, regardless of treatment. PR fetuses whose mothers were exposed to rosiglitazone in the periconceptional period had higher ANP mRNA compared to Control fetuses with maternal periconceptional rosiglitazone exposure or PR fetuses with maternal periconceptional vehicle exposure.

Conclusion: Placental restriction caused a decrease in the cardiac expression of mRNAs encoding molecules involved in proliferation in late gestation, along with a shift in the levels of mRNAs encoding metabolic enzymes that may favour fatty acid metabolism over glucose metabolism. Increasing maternal insulin sensitivity during the periconceptional period did not correct the cardiac effects of PR measured in this study, and may impose additional deficits, but only in the IUGR fetus.

HBP 54: HEMODYNAMIC CHARACTERIZATION OF 300 DAYS OLD NOD B10 FOZ/FOZ MICE

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Introduction: Obesity and diabetes are major risk factors for heart attack and are associated with hypertension, cardiac hypertrophy and cardiomyopathy. The present study investigated hemodynamic parameters and cardiac weight of fat Aussie (foz/foz) mice (obese and diabetic) studied on a mixed (NOD and B10) background.

Methods: Blood pressure (BP) and heart rate (HR) were measured by tail-cuff and carotid artery catheterization in 300 day-old male and female foz/foz and age-matched wild-type (WT) mice (n=10–11 in each group). Cardiac size and function were measured by echocardiogram. Results were expressed as mean±SEM.

Results: BP was higher while HR was lower in foz/foz mice than WT. This trend was consistent in tail cuff and carotid artery catheterization. Left ventricular (LV) diastolic pressure was similar in each group. LV dp/dt, a marker for cardiac contractility, was increased in foz/foz mice compared to WT in female mice. LV end-diastolic diameter and cardiac output (CO) were increased in foz/foz mice. LV posterior wall was thicker in male (1.16±0.04 vs. 0.98±0.06 mm) and septum was thicker in female foz/foz mice than WT (1.21±0.06 vs. 0.91±0.04 mm). Left ventricle+septum weight (LV+S)/tibia length was higher in foz/foz mice than WT mice. BW, LV+S/tibia, SBP, LV dp/dt diameter, CO (g, mg/cm, mmHg, mmHg/s, mm, ml/min, respectively) were male: WT: 39.4±1.1, 57.2±1.9, 117±3, 10280±1399, 3.75±0.14, 17.3±1.1; foz/foz: 60.3±1.4,* 78.1±2.9,* 131±3,* 12539±1054, 4.18±0.11,* 26.1±1.1;* female: WT: 33.0±2.0, 39.6±1.5, 109±2, 6673±931, 3.37±0.08, 18.9±1.4; foz/foz 56.6±2.3,* 60.4±2.8,* 129±4,* 10047±653,* 4.07±0.22,* 21.9±0.5,*; where * indicates P<0.05 vs. WT (unpaired t-test)
ISCP 01: INVESTIGATION OF THE MECHANISM(S) OF FLAVONOL-INDUCED CARDIOPROTECTION IN RAT ISOLATED HEARTS

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**Background and Aims:** The synthetic flavonol 3’,4’-dihydroxyflavonol (DiOHF) reduces myocardial ischaemia/reperfusion (I/R) injury however the mechanism of DiOHF-induced cardioprotection remains to be elucidated. The present study tested the hypothesis that DiOHF-induced cardioprotection results from selectively inhibiting injurious kinases without affecting protective kinases.

**Methods:** Langendorff-perfused rat hearts were subjected to 20 min global ischaemia followed by 30 min reperfusion in the presence of DiOHF (10 μM) or its vehicle control (0.5% dimethyl sulfoxide). Myocardial contractility and coronary perfusion pressure were continuously monitored. Lactate dehydrogenase (LDH) release into the perfusate was measured and western blotting was used to measure the expression of signalling proteins.

**Results:** Hearts treated with DiOHF showed improved cardiac relaxation [change in left ventricular –dp/dt (% of pre-ischaemia) vehicle control 41 ± 7%, DiOHF-treated hearts 70 ± 10%, p<0.05] and reduced coronary perfusion pressure (vehicle control 77 ± 2 mmHg, DiOHF-treated hearts 57 ± 7 mmHg, p<0.05) 15 min after reperfusion. The incidence of arrhythmias during the first 10 min of reperfusion also tended to be lower with DiOHF treatment (vehicle control 42 ± 10%, DiOHF-treated hearts 32 ± 12%, p=ns). LDH release, an indicator of cell death, was decreased with DiOHF treatment (vehicle control 368 ± 31 U/L, DiOHF-treated hearts 227 ± 17 U/L, p<0.01). The TUNEL assay also showed that the number of apoptotic cells after I/R was reduced with DiOHF treatment (vehicle control 35 ± 3%, DiOHF-treated hearts 22 ± 4%, p<0.05). Using western blotting, DiOHF treatment significantly reduced the I/R-induced increased phosphorylation of c-Jun N-terminal kinases (JNK, 20% decrease, p<0.05) while I/R-activated phosphorylation of p38 mitogen-activated protein kinases, extracellular signal-regulated kinases 1/2 and signal transducer and activator of transcription 3 were not affected. In addition, the phosphorylation of the multi-functional calcium/calmodulin-dependent kinase II (CaMKII) was elevated in I/R and was reduced by DiOHF treatment (25% decrease, p<0.05). The phosphorylation of the downstream target of CaMKII, phospholamban (PLN) was also reduced with DiOHF treatment (55% decrease, p<0.05).

**Conclusions:** These data suggest that the DiOHF-induced cardioprotection against I/R injury is mediated by inhibiting the phosphorylation of injurious kinases JNK, CaMKII and PLN without affecting protective kinases.

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ISCP 02: SHOCK IN PATIENTS WITH TAKOTSUBO CARDIOMYOPATHY (TTC): A ROLE FOR CALCIUM INFUSION?

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**Background and Aims:** In Takotsubo cardiomyopathy (TTC) there is emerging evidence of peroxynitrite-induced myocardial inflammation which is known to be associated with impaired systolic Ca2+ availability. Clinically, TTC is often associated with early hypotension: some patients develop shock (S-TTC), usually in the absence of pulmonary oedema. Treatment of S-TTC is conceptually difficult because of (i) contribution of catecholamines to TTC pathogenesis (ii) arterial refractoriness to catecholamines, and (iii) massive (inflammatory) release of vasodilator BNP from the myocardium. We have therefore (i) evaluated the incidence and outcome of S-TTC and (ii) conducted a pilot evaluation of effectiveness of calcium infusion (Ca-I) in reversing S-TTC.

**Methods:** We evaluated in-hospital haemodynamic status in comparison to survival in 212 consecutive patients with TTC. 5 consecutive patients with S-TTC were treated with Ca-I in a median of 12 (IQR: 5-38) hours.

**Results:** Overall, 29 developed shock during hospital stay. In the absence of shock, mortality rate was only 1%. However, in the presence of shock, mortality was increased up to 31% (p=0.001). Of the 29 patients with S-TTC, 24 were treated “conventionally” including fluid administration, catecholamine infusion and IABP insertion, with 9 (31%) in-hospital deaths. Ca-I was associated with increases in systolic BP (from 81 to 98 mmHg; p<0.05) at 6 hours, with one death following cessation of Ca-I.

**Conclusion:** (1) Overall in-hospital mortality with TTC is approximately 5%, largely associated with S-TTC. (2) Current (heterogeneous) management practices for S-TTC are associated with substantial mortality. (3) Ca-I appears to limit hypotension in S-TTC, and its impact on outcomes deserves further evaluation.

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ISCP 03: CLINICAL IMPROVEMENT OF CHRONIC HEART FAILURE COURSE AT ISCHEMIC CARDIOMYOPATHY USING IN COMPLEX TREATMENT LIPOSOMAL FORMS OF PHOSPHATIDYLCHOLINE AND QUERCETIN

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Nowadays at least 15-23 million people suffer from chronic heart failure around the world. Ischemic cardiomyopathy covers approximately 5-8% of all cases of ischemic heart disease, and between all cardiomyopathies it takes 11-13%. From all this cases men average 90%. At the way of searching of new therapy lines of chronic heart failure at ischemic cardiomyopathy we have added the liposomal transport forms of phosphatidylcholine and quercetin in a complex treatment. There were 60 patients in the study with stable clinical course during 3 months of chronic heart failure of II-IV functional classes by NYHA at ischemic cardiomyopathy, aged 20 – 49 years. By random sample patients were
ISCP 04: CURCUMIN, A P300-SPECIFIC HAT INHIBITOR, PREVENTS CARDIAC HYPERTROPHIC RESPONSES VIA EPIGENETIC MODIFICATIONS

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Background: Until recently, studies concerning histone modifications by p300 have been performed within the flexible tails, such as H3K9 and H3K14, but not the globular domain of histone. H3K122 is just reported as a novel site in the histone globular domain acetylated by p300. We previously demonstrated that curcumin, a p300 histone acetyltransferase inhibitor, prevents the acetylation and transcriptional activity of GATA4 as well as pathological cardiomyocyte hypertrophy and the development of heart failure in vivo. However, it is still unclear whether hypertrophic stimulation induces the histone modifications and these changes are suppressed by curcumin.

Hypothesis: We hypothesized that curcumin could inhibit p300-induced acetylation of the globular domain as well as tail domain of histone during the development of cardiac hypertrophy in vitro and in vivo.

Methods and Results: Cultured cardiomyocytes prepared form neonatal rats were stimulated with or without phenylephrine (PE) for 48 h. Nuclear extracts and histones prepared from these cells were subjected to Western blotting (WB). Treatment with PE increased both the acetylation of H3K122 and those of H3K9 and H3K14 in cardiomyocytes. Chromatin-immunoprecipitation (ChIP) assays demonstrated that PE increased the recruitment of acetylated H3K122 and H3K9 onto ANF and BNP promoters containing the GATA element. Treatment with curcumin significantly inhibited these changes. Next, to investigate the role of Curcumin in vivo, we utilized the salt-sensitive Dahl rats (DS). Six-week-old DS (n=19) and control salt-resistant Dahl rats (DR, n=10) were given a high-salt diet and randomly assigned to daily oral treatment with 50 mg/kg/day of curcumin or its vehicle for 6 weeks. After treatment (at 12 weeks of age), the left ventricular (LV) wall thickness, LV mass and LV fractional shortening were significantly higher in DS compared with DR, and the LV end-systolic and end-diastolic dimensions were significantly smaller in DS than in DR. Curcumin treatment significantly (p<0.001) decreased the LV wall thickness in DS, but not in DR. Curcumin also significantly (p<0.001) decreased the LV mass in DS. Acetylated H3K9 and H3K122 in the hearts were increased in DS compared with DR. Curcumin significantly inhibit these acetylations.

Conclusion: The natural compound curcumin inhibits not only the tail domain but also the globular domain of histone in vitro and in vivo and the development of hypertension-induced concentric LVH. Thus, this compound might be applicable to patients with hypertensive heart diseases.

ISCP 05: ANALYSIS OF NOBEL TARGET MOLECULES OF NOBILETIN, A POTENT THERAPEUTIC AGENT AGAINST HEART FAILURE

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Introduction: Heart failure is a syndrome associated with increasing prevalence, high mortality, and frequent hospital admissions and imposes a significant economic burden on healthcare systems of industrial countries that is expected to further increase in the future due to the ageing population. Maladaptive hypertrophy is being recognized as a critical event during the development of heart failure. We screened a natural compound library and found that nobiletin, a poly-methoxy flavonoid derived from Citrus unshu, repressed phenylephrine (PE)-induced hypertrophic responses in cardiomyocytes. In a previous study, we demonstrated that nobiletin prevented the deterioration of systolic function and LV pathological hypertrophy after myocardial infarction in rats. Thus, nobiletin is expected as an attractive pharmacological agent for the treatment of heart failure. However, the target molecule of nobiletin in cardiomyocytes is still unclear.

Hypothesis: We hypothesized that proteomics analysis can be used to identify novel nobiletin-binding proteins (NBPs) with an anti-hypertrophic effect.

Methods and Results: First, the intracellular distribution of nobiletin in cardiomyocytes was investigated by generating nobiletin conjugated with Tokyo-green (TG-nobiletin), a fluorescein analogue. TG-nobiletin was observed in the cytoplasm of cardiomyocytes, and it retains the ability to inhibit cardiomyocyte hypertrophy as well as original nobiletin. Next, to purify NBPs, biotin-conjugated nobiletin (Bio-nobiletin) was synthesized. Protein extracts form rat hearts were incubated with Bio-nobiletin or Biotin alone and binding proteins were precipitated with streptavidin beads.
mass spectrometric analysis, we identified 162 novel NBPs including several intracellular kinases, HDAC, and unknown proteins. One of them was NBP1, involved in metabolic processes and mitochondrial functions. Pull-down assays confirmed that Bio-nobiletin could directly interact with recombinant NBP1. In cardiomyocytes, knockdown of NBP1 blocked the inhibitory effect of nobiletin against PE-induced cardiomyocyte hypertrophy. Conversely, overexpression of NBP1 inhibited PE-induced hypertrophy and increased mRNA levels of mitochondrial- and lipid metabolism-related gene transcriptions, such as PGC1α, MFN2, PPARα/γ, mCPT1, and MCAD.

Conclusions: Proteomics analysis of molecular targets of nobiletin is useful to delineate signaling pathways that mediate hypertrophic responses in cardiomyocytes. NBP1 may be a molecular target of nobiletin, which is a potent therapeutic agent for heart failure.

ISCP 06: SEX DIFFERENCES IN VASOMOTOR REACTIVITY
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Background and aims: Worse clinical outcomes in female patients with and without established coronary disease have been reported. Although multiple mechanisms are implicated, vascular reactivity has not been examined. Sex-dependent differences in vasoreactivity were assessed in isolated large- and micro-vasculature including: 1) internal mammary artery (IMA), 2) saphenous vein (SV) and 3) subcutaneous arteries (=250μm) from patients undergoing CABG and elective non-cardiac surgery.

Method: Vascular wire myography was used to determine concentration-response curves from which half maximal effective concentration (EC50) was calculated for; endothelin-1 (Et-1), phenylephrine (PE), serotonin (5HT) and the thromboxane-mimetic, U46619.

Results: Compared with men, women undergoing CABG demonstrated increased constrictor responses to PE and 5HT in the IMA but no difference in SV (data not shown) or subcutaneous arteries. In contrast, subcutaneous arteries in women without a history of cardiovascular disease (healthy controls) show increased sensitivity to U46619, 5HT and PE, compared with men. *p < 0.05.

<table>
<thead>
<tr>
<th>EC50 (Mean±SEM)</th>
<th>Coronary Artery Disease (undergoing CABG; n=80)</th>
<th>Controls (undergoing non-cardiac surgery; n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculature</td>
<td>IMA Large vessel segments</td>
<td>Subcutaneous microvessels</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Et-1 (nM)</td>
<td>11.9±1.7</td>
<td>12.9±2.2</td>
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<tr>
<td>U46619 (nM)</td>
<td>6.5 ± 1.2</td>
<td>3.4±0.9</td>
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<tr>
<td>5HT (μM)</td>
<td>0.8 ± 0.3</td>
<td>0.1±0.03*</td>
</tr>
<tr>
<td>PE (μM)</td>
<td>3.8 ± 1.8</td>
<td>1.1 ± 0.3*</td>
</tr>
</tbody>
</table>

Mechanistic studies evaluating female hypersensitivity to PE in IMA segments, demonstrated (1) no sex difference in total α1 and β2 adrenoreceptor abundance by Western blot analysis, (2) persistence of the observed sex-difference following nitric oxide synthase inhibition by L-NAME, (3) the cyclooxygenase inhibitor indomethacin abolished the sex difference in vascular reactivity.

Conclusion: For the first time, female vascular hyper-reactivity in both large graft arteries (IMA) and microvessels has been demonstrated. In part, this may be due to sex-differences in prostanoid activity. The IMA hyper-reactivity in women may contribute to their poorer outcomes following CABG and microvascular differences amongst patients without documented cardiovascular disease may pre-dispose them to hypertension.

ISCP 07: CURRENT ANTIHYPERTENSIVE DRUG THERAPY IN SWEDEN IN RELATION TO GENDER, AGE AND COMORBIDITY
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Background and Aims: To describe current antihypertensive drug therapy in Sweden in relation to gender, age and comorbidity.

Methods: By use of the Stockholm regional healthcare data register, comprising all healthcare consultations, hospitalizations and dispensed drugs for 2.1 million inhabitants in the Greater Stockholm region, we identified all persons 20 years or older with a recorded diagnosis of hypertension during 2009-2013, their comorbidity, and their dispensed antihypertensive drugs during 2013.

Results: We identified 292 623 patients aged 20–109 (mean 63) years, 154 230 (53%) were female. The most common comorbidities in females were diabetes (17%), COPD (17%) and ischemic heart disease (11%), and in males diabetes (24%), ischemic heart disease (17%) and atrial fibrillation (13%). The most common antihypertensive drug classes in females and males were beta blockers (39 and 38%; P<0.01), calcium channel blockers (29 and 34%; P<0.001), ACE
by professionals treating them. The practice is undergoing slowly. The goal of this study was to analyse main problems of OAC clinical usage by patients with NVAF and causes many problems for patients and physicians. Novel OAC promise to solve those problems; however, their implementation in

Background:
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ANTICOAGULANTS

ISCP 09: DIFFERENT CLINICAL ASPECTS IN REAL PRACTICE IN PATIENTS WITH ATRIAL FIBRILLATION AND ORAL ANTIICOAGULANTS

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Background: Old generation oral anticoagulants (OAC) have been first line medication for prevention of thromboembolic events by patients (pts) with non-valvular atrial fibrillation (NVAF) for a long time, although the usage of vitamin K antagonists (VKA) causes many problems for patients and physicians. Novel OAC promise to solve those problems; however, their implementation in practice is undergoing slowly. The goal of this study was to analyse main problems of OAC clinical usage by patients with NVAF and by professionals treating them.

Methods: The study enrolled 2631 pts with NVAF under OAC therapy in different Latvian university and regional hospitals and ambulatory praxis. Problems associated with OAC, side effects and interactions, awareness of patients, complexity of OAC usage were analysed from patient perspective. Events of bleeding were defined as Clinical Relevant Major Bleeding (CRMB) and clinical relevant non-major bleeding (CRNMB) according to international guidelines. Second study group included 245 medical practitioners with clinical experience in treatment and care of non-valvular AF patients applying OAC. Difficulties during the treatment and choice of OAC were analysed from the physician's point of view.
Results: There were 1866 (70.9%) users of VKA and 765 (29.1%) users of novel OAC (NOAC). According to CHA2DS2-VASc, in VKA group, scale median was 3, in NOAC group – it was 2.5. Significantly higher incidence of side effects was detected among VKA users compared to novel oral anticoagulants (NOAC) users. All cases of bleeding were reported: 31% in VKA vs 3.3% in NOAC users (p<0.001); CRMB in VKA gr:had 32 pts (1.7%) vs no CRMB were observed in NOAC (564 which included 652 dabigatran and 113 rivaroxaban). CRNMB in VKA group had 164 pts (8.85%) vs 12 (1.6%) in NOAC. Less than a half of the patients followed the interaction of active substances with OAC, besides, patients were less informed about this aspect compared to OAC side effects and INR controls in VKA group. More than 50% of the VKA users had difficulties to adjust OAC dose and to keep the INR between 2.0 and 3.0 and 31.8% had problems with INR control despite the fact that 90.6% were regular undergoing INR control, mostly one to two times a month. Dabigatran was preferred in patients in electrical cardioversion group 64.7% vs. 35.3% VKA with significantly lower rates of adverse events (p<0.001) as bleeding and high safety by neurological aspects in long term control.

In physicians’ group: 13.9% cardiologists, 20.8% internal specialities, 23.8% general practitioners, 8.9% surgeons.

ISCP 10: COMPARISON OF EFFICACY AND SAFETY OF TWO DOSAGES DABIGATRAN VERSUS WARFARIN IN PATIENTS WITH PERSISTENT AND LONG-STANDING ATRIAL FIBRILLATION UNDERGOING ELECTRICAL CARDIOVERSION

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Introduction: The most important factor for efficacy and safety for patients with atrial fibrillation (AF) undergoing electrical cardioversion (ECV) is appropriate use of oral anticoagulant (OAC) therapy. Dabigatran is a possible alternative OAC therapy before and after ECV versus therapy with warfarin.

Methods: We have analysed the data collected before, during and after ECV in 1046 patients (pts) undergoing ECV. All pts had AF, 885 defined as persistent and 161 defined as long-acting, mean CHA2 DS2 VASc score was 3.1 ± 1.8, 735 had one or two ECV in anamnesis. 628 (60%) pts started the use of dabigatran, (405 pts 150 mg twice or 223 pts 110 mg twice) before ECV for at least 21 day, 418 (40%) started warfarin therapy and 21 day start after INR was in range 2.0 – 3.0. 110 mg twice were prescribed for pts ≥ 75 year old, HASBLEED risk score and kidney problems. Transesophageal echocardiography (TEE) was encouraged before ECV in all groups for pts with CHA2DS2VAsc score ≥ 3, markedly left atrial dilatation and AF duration ≥ 6 months. ECG and Echo-kg data were analysed 30 and 90 days after ECV.

Results: ECV was successful after first shock in 962 (91.7%) pts, in total successful ECV – in 1029 (98.37%) pts. Left atrial thrombi were detected on TEE before ECV in 7 pts in dabigatran group and 12 pts in warfarin group, so, pts continued OAC therapy for one month, and TEE had been performed again after. 3 pts in dabigatran (150 mg twice) group and 2 pts in warfarin group were free of thrombus and have been referred to ECV. Average time for treatment before ECV was significantly lower for dabigatran (25 days) vs warfarin (44 days, p<0.01). Stroke and systemic embolism rates at 90 days were lower in both dabigatran group (0.1%) vs warfarin group (1.4%), but the event in both dabigatran group was documented after discontinuation of the drug while 3 warfarin events were detected under the time of use of OAC. There was no difference in analysis of events between TEE and non-TEE pts.

Dabigatran pts had significantly lower clinical relevant bleeding rate vs warfarin (D 110 mg 0, D 150 mg 0.47% vs W 2.87%, p<0.04) and better compliance.

Conclusions: Dabigatran 150 mg and 110 mg twice is a safe, effective and reasonable alternative to warfarin for patients undergoing ECV despite CHA2DS2VAsc risk score, HASBLEED score and AF duration. The frequencies of stroke and embolic events were lower in dabigatran 150 mg and 110 mg versus warfarin with lower major bleeding within 30 and 90 days after ECV. Patients undergoing dabigatran therapy have shorter time before procedures.

ISCP 11: RISK FACTORS AND INCIDENCE OF LIFE THREATENING VENTRICULAR ARRHYTHMIAS AND ITS IMPACT ON CLINICAL OUTCOMES IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Introduction: Life threatening ventricular arrhythmias (LTVA) are rare acute coronary syndrome (ACS) complication but with a significantly high mortality rate. At the moment, there are no defined risk factors of LTVA. Assess the rate of LTVA in patients with ACS and its types (ACS with ST segment elevations (ACS STEMI) and ACS without ST segment elevation (ACS NSTEMI)), the impact of the treatment on the rate of LTVA, as well as assessing the possible risk factors for the development of LTVA and its impact on clinical outcomes.

Methods: Data from Latvian Acute Coronary syndrome register and data from patients, who have been hospitalized in Pauls Stradins Clinical University Hospital 32nd invasive cardiology ward in the year 2011 and 2012. Descriptive statistical methods were used.

Results: LTVA rate – ACS 4.2%; ACS STEMI 9.5%, ACS NSTEMI 2.0%. In ACS STEMI patients undergoing primary percutaneous coronary intervention (PPCI), LTVA rate is 12.3%, but in patients with fibrinolysis – 9.5% (p=0.95). Intrahospital mortality rate was higher in patient group with LTVA 28% vs 4.7% in patients without LTVA, p<0.001; ACS STEMI 28.4% vs. 7.6%, p<0.001; ACS NSTEMI 27% vs. 3.6%, p<0.001. In the group of patients who didn’t have LTVA, there was a higher rate of arterial hypertension (AH) diagnosis (69.6% vs.49%, p<0.001), previous PCI (22.1% vs.12.9%, p=0.036), congestive heart failure (CHF) (35.2% vs.20.4%, p=0.004), previous use of beta adrenergic blockers (BAB) (27.2% vs.16.1%, p<0.001; ACS NSTEMI 27% vs. 3.6%, p<0.001. In the group of patients who didn’t have LTVA, there was a higher rate of arterial hypertension diagnosis (69,6% vs.49%, p<0.001), previous PCI (22.1% vs.12.9%, p=0.036), congestive heart failure (CHF) (35.2% vs.20.4%, p=0.004), previous use of beta adrenergic blockers (BAB) (27.2% vs.16.1%,
ISCP 12: RAPID DESENSITIZATION OF NEUTROPHILS TO ANTI-INFLAMMATORY EFFECTS OF BNP: IMPLICATIONS IN TAKO-TSUBO CARDIOMYOPATHY

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Background and aims: We have previously demonstrated that B-type natriuretic peptide (BNP) inhibits the release of superoxide anion (O2-.) from neutrophils and this effect is attenuated in acute heart failure (AHF) (in association with pronounced distensive BNP release). Tako-tsubo cardiomyopathy (TTC) is an acute inflammation of myocardium induced by catecholamine release and characterized by “inflammatory” BNP release. We sought to compare impact of TTC and AHF on BNP/neutrophil interactions.

Methods: Neutrophil O2- production was quantitated utilizing EPR spectroscopy with CMH as the spin trap. Inhibitory effects of BNP were determined by pre-incubation of neutrophils with BNP (1μM) then stimulation by either phorbol 12-myristate 13-acetate (PMA, 100nM) or N-formyl-methionyl-leucyl-phenylalanine (fMLP, 1μM). We compared (i) healthy subjects (n=29), (ii) AHF patients (n=45), and (iii) TTC patients (n=15). In order to evaluate the time course of putative change in BNP response in TTC, we correlated duration of symptoms and plasma NT-proBNP levels with residual BNP response.

Results: Among control subjects, independent of age/gender, BNP inhibited neutrophil O2- release by 25-30%. This was similarly attenuated (P<0.05) in both AHF and TTC patients (Figure 1). Among TTC patients (mean peak NT-proBNP levels was 4700±500pg/ml), there was no correlation between peak NT-proBNP levels and BNP response; duration of symptoms (median 3 days) was not a significant determinant of BNP response.

Conclusions: TTC is associated with intense myocardial inflammation and BNP release. Paradoxically, this results in rapid suppression of the BNP-mediated anti-inflammatory response, potentially aggravating/prolonging myocardial dysfunction. Potentially desensitization to BNP in AHF induces similar changes.

ISCP 13: HOMO-ARGININE INCREASES TISSUE ROS PRODUCTION BUT DOES NOT AFFECT PLATELET AGGREGATION

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Background and aims: Homanarginine (HA) is present in human plasma in concentrations of approximately 2μmol/L. While structurally related to arginine, HA appears to be minimally active as a nitric oxide (NO) synthase substrate. Inferential evidence suggests that HA may not be inert: recent studies showed that low plasma [HA] represents an independent risk factor for heart disease and there are emerging data that it may be pro-oxidant.

Methods: We evaluated the effects of HA on (1) reactive oxygen species (ROS) release from H9c2 myocytes and (2) aggregation responses to adenosine 5'-diphosphate (ADP) in whole blood, a process critically modulated by both endogenous and exogenous NO. ROS release was quantified utilizing electron paramagnetic resonance spectroscopy with CMH as the spin trap. Aggregation was determined utilizing 2.5μM ADP and a whole blood impedance aggregometer (Model 560, ChronoLog Corporation); responses were recorded as electrical impedance in Ohms (Ω).

Results: In H9c2 myocytes, HA (50μM) increased ROS release rate from 0.5±0.01×106 to 0.8±0.1×106 (p<0.05) with threshold effects at or below 5μM and a local maximum at 50μM (see Figure). Arginine (100μM) induced minimal changes. In human whole blood, mean platelet response to ADP was 5.9 ± 0.6Ω in the absence of and 5.6 ± 0.5Ω in the presence of 0.5μM HA (p=NS).

Conclusions: HA, in physiologically relevant concentrations, increases ROS release in H9c2 cells. (2) On the other hand, HA has no significant impact on the extent of human platelet aggregation. These data provide further evidence of a pro-oxidant effect of HA, an effect which is discordant with its reported impact on patient survival.

ISCP 14: SDMA: NOT SO INERT!

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Background and aims: Symmetric dimethylarginine (SDMA) is a methylated arginine derivative enzymatically generated by protein catabolism and cleared both metabolically and via renal secretion. While other methylated
arginine derivatives, such as the closely related asymmetric dimethylarginine (ADMA) function primarily as nitric oxide synthase inhibitors, SDMA has been assumed to be biologically inert. Recent findings have, however, suggested: (1) elevated SDMA levels predict cardiac mortality rates in a wide variety of populations; (2) SDMA may exert pro-oxidant effects. We sought to determine whether SDMA might affect reactive oxygen species (ROS) production and/or platelet aggregation, both reactive oxygen species-sensitive processes.

**Methods:** We evaluated the effects of SDMA on ROS release from cultured H9c2 myocytes and whole blood aggregation responses to adenosine 5'-diphosphate (ADP) in healthy subjects. ROS release was quantified utilizing electron paramagnetic resonance spectroscopy with CMH as the spin trap. Aggregation was determined utilizing 2.5μM ADP and a whole blood impedance aggregometer (Model 560, ChronoLog Corporation). Responses were recorded as electrical impedance in Ohms (Ω).

**Results:** SDMA in concentrations from 5 to 1000μM increased ROS production by H9c2 cells by 15 to 50% (p<0.01, ANOVA). On the other hand, ADMA induced no marked increases. Furthermore, SDMA (30μM) significantly increased ADP-induced aggregation (p<0.01).

**Conclusions:** These data provide further support to the idea that SDMA is a pro-oxidant, which increases tissue ROS release. Furthermore, it exerts a pro-aggregant effect which may contribute to its association with cardiovascular risk.

**References:**

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**ISCP 15: AN AUDIT OF THE USE OF EPLERENONE POST STEMI WITH PRIMARY PCI AT UNIVERSITY HOSPITAL BIRMINGHAM**

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**Purpose:** The EPHESUS trial (2003) showed there was a significant improvement in mortality when using Eplerenone within 3 – 14 days following STEMI in patients with a poor LVEF and signs of heart failure. NICE then suggested its use in such patients in its 2007 and updated 2013 guidance. We wanted to audit the use of Eplerenone in our cardiology department and determine whether we were adhering to NICE guidance with the aim of highlighting systemic causes for deviation.

**Methods:** The 214 patients who were treated with primary PCI for STEMI in 2013 had their clinical documentation reviewed; chest X-ray, medication e.g. diuretic use and discharge documentation from the hospital’s electronic database were used to ascertain whether these patients had clinical evidence of heart failure in addition to ECHO diagnostic criteria for LVEF < 40%.

**Results:** Patients included in the audit met strict criteria including LVEF <40% and clinical signs of heart failure. Contraindications to treatment with eplerenone led to 5 patients being excluded. This audit found that the use of Eplerenone in the participating hospital was inconsistent. In patients who were fully eligible according to NICE guidance (n=13), there was a significant underuse of eplerenone with only 4 patients prescribed. In addition, of 23 patients who had LVEF < 40% but no signs of heart failure, 13 were unnecessarily prescribed eplerenone. Analysis of the systems found that echocardiograms were generally performed & reported 72 hours post PCI. 80% of discharge documentation was performed by junior staff. Out of 15 cardiology juniors (FY1 – CT2) questioned, 6 were aware that eplerenone should be used in post STEMI patients with poor LVEF, but none were aware that the guidelines specify that there should be poor LVEF & clinical signs or symptoms of heart failure. **Conclusions:** Inconsistencies in the prescription of eplerenone can be attributed to the lack of awareness and knowledge amongst the junior team who have final responsibility of writing and providing discharge medication. This may be in part due to poor communication between the junior and senior members of the team and a lack of trust guidance. This audit has highlighted the need for junior staff’s continued education regarding novel drugs in heart failure, which should be carried out ideally following the creation of guidance specific to the local population.

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**ISCP 16: INTERACTION BETWEEN EET- AND NO-MEDIATED ENDOTHELium-DEPENDENT DILATION OF ARTERIES FROM OBESE AND NON-OBESE RATS**

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Endothelium-dependent dilation of arteries is an important physiological function controlling blood pressure and flow. Previous studies have demonstrated the activity of nitric oxide (NO), a key mediator of endothelium-dependent vasodilation, is altered in conditions featuring cardiovascular dysfunction, such as obesity (Howitt et al., 2012). The current study investigated the interaction between NO and another proposed mediator of endothelium-dependent vasodilation, epoxyeicosatrienoic acids (EETs) in muscle arteries from rats with diet-induced obesity. Male Sprague-Dawley rats (7 – 8 weeks) were fed a cafeteria-style high fat or control diet for 16 – 20 weeks. Following anesthesia with sodium thiopentone (100 mg/kg i.p.), studies were performed in isolated, pressurized (70 mmHg) first-order arterioles from the cremaster muscle. Control rats weighed 570 ± 7 g compared with obese rats 768 ± 13 g (p=35 – 37 of each, P<0.05). Diet-induced obesity had no effect on endothelium-dependent, acetylcholine (ACH; 1nM – 3 μM)-induced dilation of arterioles. In arteries from control-diet rats, ACH-induced vasodilation was decreased by 12.2 ± 3.9% following inhibition of NO effects (NO synthase inhibitor L-NAME 100 μM plus guanylate cyclase inhibitor ODQ 10 μM), and by 17.6 ± 4.1 and 12.4 ± 3.1% in the presence of either the cytochrome P450 inhibitor 17-ODYA (20 μM) or the EET antagonist 14,15-EEZE (10 μM) respectively (n = 4 for all). The combination
of L-NAME, ODQ and 17-ODYA or L-NAME, ODQ and 14,15-EEZE abolished ACh-induced vasodilation. The soluble epoxide hydrolase (sEH) inhibitor AUDA (1 µM) alone did not alter responses to ACh. In vessels from obese rats, the NO-mediated component of the ACh effect was increased (31.1 ± 5.6%, n = 8, P<0.05, 2-way ANOVA) as reported previously (Howitt et al., 2012); the combination of L-NAME, ODQ and 17-ODYA or L-NAME, ODQ and 14,15-EEZE still abolished ACh-induced vasodilation. These studies suggest that EETs are a vital mediator of endothelium-dependent vasodilation in the rat cremaster muscle artery and the EET pathway may offer multiple targets for potential therapeutic agents.


**ISCP 17: INVESTIGATING A NANOMEDICINE APPROACH TO OPTIMIZE STATIN-BASED PHARMACO-THERAPY AGAINST CORONARY ARTERY DISEASE**

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**Introduction:** Coronary artery disease (CAD) represents the leading cause of sudden death in Australia. Statins, or HMG-CoA reductase inhibitors, have been clinically proven and prescribed to reduce coronary events and prevent CAD, among which lovastatin (LOV) is the first FDA approved statins. However, due to the low water solubility and the extensive intestinal metabolism, oral bioavailability of conventional LOV formulations available on the market is low and erratic (< 5%). The current study aims to design and develop a nanomedicine approach to address the issues limiting the oral therapeutic efficacy of LOV and to develop an improved pharmaco-therapeutic approach for CAD prevention.

**Methods:** Two LOV-loaded silica-lipid hybrid (SLH) were fabricated by spray drying from a self-emulsifying lipid (Gelucire® 44/14) and two types of silica, specifically Aerosil® 380 fumed silica nanoparticles (SLH-A) and Syloid® 244FP porous silica microparticles (SLH-S). In vitro drug solubilization studies were conducted under simulated intestinal conditions (5mM bile salt: 1.25mM phospholipids and 1000 TBU/ml pancreatic lipase at 37°C), followed by a re-dissolution study of post-digestion precipitates at pH 6.8, 37°C. Single-dose pharmacokinetics (PK) of LOV was determined in fasted rats at 5mg/kg.

**Results and Discussion:** The SLHs appeared to be white agglomerated powders, which were easy to handle and allow effective formulation into tablets. LOV was encapsulated in its non-crystalline form at 10% w/w in SLH-A and SLH-S, which is 3-times higher than the drug loading into Gelucire lipid alone, and up to 10-fold higher than the loading levels reported with other lipid-based carrier for LOV, i.e., <1% in dry emulsions. Furthermore, the SLHs were shown to improve drug solubilization under simulated intestinal condition by at least two fold; generate potential transient supersaturation in the aqueous intestinal micro-environment due to rapid re-dissolution of amorphous precipitates; and consequently, contribute to up to 2.8-fold improvement in the oral bioavailability in comparison to pure LOV.

**Conclusion and Future Perspective:** Overall, the nanostructured SLH carriers provide a promising formulation strategy to overcome the solubility-limited delivery challenges associated with the oral use of LOV. In the next step, the SLH approach will be further explored for the combined delivery of LOV and another anti-atherogenic agent (e.g. nitric oxide-donor) to provide synergistic activity against atherosclerosis, and hence a more efficient statin-based pharmaco-therapeutic approach.

**ISCP 18: IMPROVING SIGNALLING FIDELITY IN AGED HEARTS: CAVEOLIN 3 LIMITS AGE-RELATED ISCHAEMIC INTOLERANCE**

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With age, the heart becomes more susceptible to stress and less responsive to therapeutic interventions aimed at protecting against ischemia-reperfusion (adenosine, opioids, ischemic preconditioning etc.). Age-dependent dysfunction in myocardial stress-signaling fidelity and metabolic flexibility may involve loss of ‘scaffold’ co-ordination of cellular signalling. We examined the contribution of caveolin-3 (Cav-3), a scaffolding protein, to cardiac ischaemic tolerance in young to aged mice. We assessed age-dependent ischaemic intolerance in male C57BL/6 mice aged 8, 16, 32 and 48 weeks of age. Animals were anaesthetised with Inactin (125 mg/g body weight, i.p.), and hearts removed and hearts perfused in a Langendorff mode with an intraventricular balloon for assessment of contractile function. Hearts underwent 20 minutes of ischaemia followed by 45 minutes of reperfusion. Mice at 8-weeks of age recovered ~55% of pre-ischaemic function, with significantly elevated diastolic pressure (~19mmHg) and evidence of cell death (~5.4µg/g cardiac Troponin release). Recovery in hearts of males aged 16 weeks was not statistically different, while hearts from mice aged 32 and 48 weeks displayed significant depression of intrinsic resistance to ischemia-reperfusion injury. Decreased mRNA expression of Cav-3 that was evident by 48-weeks of age (relative to 8-week heart 0.35), and proteomic analysis revealed ~50% lower Cav-3 protein at ~2 years of age and loss of caveolar morphology. We also demonstrated loss of Cav-3 dependent protective responses (ischaemic preconditioning, adenosine receptor agonism) in older hearts. Hearts from young (3 month) and aged (18 month) mice overexpressing Cav-3 (TG) were found to be more tolerant of ischaemic insult: in young wild-type (WT) littermates, cardiac contractile recovery from ischaemia was 47% of pre-ischaemic function (with diastolic pressure elevated to 35mmHg), while recovery of contractile and diastolic function was impaired in aged WT hearts (21% and 58mmHg, respectively); Cav-3 TG hearts were significantly protected, with diastolic dysfunction reduced to 22mmHg in young TG mice and 32mmHg in aged TG mice, and ventricular pressure development recovered to 60-65% of baseline in young and aged TG hearts. These data suggest that Cav-3 plays a pivotal role in the cardiac
ISCP 19: AN ANALYSIS OF THE EFFECTIVENESS OF INVESTIGATIVE PROCEDURES FOR THE DIAGNOSIS OF CLOZAPINE-INDUCED MYOCARDITIS


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Background and aims: A comprehensive analysis of investigative methods for diagnosis of clozapine-induced myocarditis has not previously been conducted. Data from a case-control study were available to be used for this purpose.

Methods: 109 cases and 334 controls were documented from medical records with recording of results of all diagnostic procedures and follow-up investigations. Cases were confirmed using a case definition.

Results: Investigations with sufficient data for analysis were troponin I/T, echocardiography, creatine kinase, creatine kinase-MB, C-reactive protein (CRP), erythrocyte sedimentation rate and erythrocyte counts. The best diagnostic performance (88% cases positive; 98% controls negative) for clozapine-induced myocarditis was found with troponin (threshold ≥2 upper limit of normal – ULN). CRP (threshold 100mg/L) also performed well with sensitivity and specificity of 76% and 84%, respectively. All other investigations had a poor diagnostic performance and their role as a primary diagnostic parameter could not be supported.

Of three cases with B-type natriuretic peptide results, only one tested positive (threshold 100 pg/mL), even though one of the other cases had a troponin result of 11.3 (ULN 0.04) µg/L. The case with the highest troponin (41.6; ULN 0.6 µg/L) had a normal echocardiogram and minimal rise in CRP (16 mg/L), while the case with the most severe left ventricular impairment by echocardiography (ejection fraction 10-20%) had a moderate troponin level (1.84; ULN 0.3 µg/L). The rise in troponin in the three fatal cases with results did not indicate life-threatening illness. None of the nine fatal cases underwent an assessment of cardiac function by echocardiography or cardiac magnetic resonance.

Conclusions: No single diagnostic method should be relied upon for diagnosis and severity assessment of clozapine-induced myocarditis. The most effective strategy, based on current data, is to monitor troponin and CRP serially and conduct echocardiography if either or both are elevated.

ISCP 20: CAN ORAL VITAMIN D PREVENT THE CARDIOVASCULAR DISEASES AMONG MIGRANTS IN AUSTRALIA? PROVIDER PERSPECTIVE USING MARKOV MODELLING

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Objectives: To model the effectiveness and cost effectiveness of oral Vitamin D supplementation as a primary prevention strategy for cardiovascular disease among a migrant population in Australia.

Setting: Community Health Service, Kensington, Melbourne, Australia.

Participants: Adult (19-99 years) migrants (African origin) who were vitamin D deficient and free from cardiovascular disease visiting the medical centre at least once during the period from 1st January 2010 to 31st December 2012.

Main outcome measures: The blood pressure-lowering effect of vitamin D was taken from a published meta-analysis and applied in the Framingham 10 year cardiovascular risk algorithm (with and without oral vitamin D supplements) to generate the probabilities of cardiovascular events. A Markov decision model was used to estimate the provider costs associated with the events and treatments. Uncertainties were derived by Monte Carlo simulation.

Results: Vitamin D oral supplementation (1,000 IU/d) for 10 years could potentially prevent 31 (IQR 26 to 37) non-fatal and 11 (IQR 10 to 15) fatal cardiovascular events in a migrant population of 10,000 assuming the 100% compliance. The provider perspective incremental cost effectiveness per year of life saved was AUS 3,992 (IQR 583 to 8558).

Discussion: This model assumed the cost of the intervention is being generated by the provider and it is free for the patients. In spite of the treatment cost the model has showed the intervention to be highly cost-effective. At present oral vitamin D is not subsidized by the Pharmaceutical Benefit Scheme. Therefore it is worth considering subsidization of oral vitamin D (vitamin D3, 1000 IU as a maintenance daily dose) in this high risk population group.

Limitations: The probabilities of the main outcomes were generated using the Framingham 10 year cardiovascular disease risk prediction algorithm which was originally developed from an urban US, mainly Caucasian, population. As our study population consisted of migrants of African origin, it could give rise to a source of error in our estimations.

Conclusion: This study suggests subsidized supplementation of oral vitamin D may be a cost effective intervention to reduce non-fatal and fatal cardiovascular outcomes in high-risk migrant populations.

ageing process, and suggest that augmenting Cav-3 expression may eliminate age-related ischemic intolerance. Thus, therapies that elevate Cav-3 expression in the heart may be a novel means to limit cardiac aging.
**ISCP 21: STATINS DECREASE SERUM AMYLOID A- AND ALPHA1-ANTITRYPSIN-LDLs, OXIDATIVELY MODIFIED LDLs THAT ACCELERATE ATHEROSCLEROSIS**

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**Introduction:** Statins, HMG-CoA reductase inhibitors, reduce the risks of death due to cardiovascular diseases. Statins exhibit pleiotropic effects on inflammation, oxidative stress, and endothelial functions. In a JUPITER study, it was reported that the rate of newly diagnosed diabetes was increased in a rosuvastatin (RSV)-treated group. However, the relationships between the effects of statins on inflammation and oxidative stress and those on diabetes are largely unknown. Two types of oxidatively modified low-density lipoprotein (LDL), serum amyloid A (SAA)- and α1-antitrypsin (AT)-LDLs, are involved in the progression of atherosclerosis. We therefore investigated: (i) the effect of statins on SAA- and AT-LDLs, and (ii) the relationship between statin’s effects on these LDLs and those on diabetes.

**Methods:** The Institutional Review Board at the National Hospital Organization Kyoto Medical Center approved this study. All subjects provided written informed consent prior to participation. This study is a sub-analysis of the STAT-LVDF study, an open-label, randomized, parallel comparative prospective study. The subjects received the treatment with RSV or pitavastatin (PTV) for 24 weeks. LDL-cholesterol levels of the patients were controlled by these statin treatments according to the guideline.

**Results:** A total of 53 patients were analyzed for this study. There was no significant difference in the sex distribution or age between the RSV group and in changes in the body mass index, heart rate, or HbA1c by treatment with RSV and PTV. Statin treatment significantly reduced LDL-C and TG (187 ± 123 vs. 156 ± 116 mg/dL, p=0.018, in the RSV group, 195 ± 97 vs. 145 ± 82 mg/dL, p<0.001, in the PTV group). Statin treatment significantly decreased SAA-LDL (7.0 vs. 5.0 μg/mL, p=0.003, in the RSV group, 7.5 vs. 5.0 μg/mL, p=0.012, in the PTV group) and AT-LDL (1.5 vs. 1.3 μg/mL, p=0.013, in the RSV group, 1.4 vs. 1.3 μg/mL, p=0.037, in the PTV group) levels.

The change ratio of SAA-LDL by statin treatment was closely correlated with that of CRP (p=0.003, in RSV, p=0.004 in PTV, p<0.001 in all). Interestingly, the change ratio of SAA-LDL was correlated with that of HbA1c (p=0.030) in the PTV group, but not in the RSV group.

**Conclusion:** This study revealed that both RSV and PTV reduce SAA-LDL and AT-LDL, oxidatively modified LDLs, and that a decrease of SAA-LDL is closely associated with CRP. The results suggest that the effect of statins on diabetes is associated with the anti-inflammatory effect in PTV.

**ISCP 22: EFFECTS OF PERHEXILINE ENANTIOMERS ON ACTIVATION OF MYOCARDIAL PYRUVATE DEHYDROGENASE IN A MODEL OF ISOPrenaline-INDUCED STRESS**

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**Background and Aims:** Perhexiline, a chiral drug, is an effective myocardial metabolic agent that is thought to improve energetics via inhibition of carnitine palmitoyltransferase-1, shifting myocardial metabolism from fatty acid to carbohydrate utilisation, as described by the Randall cycle. Indeed, perhexiline was shown to activate murine myocardial pyruvate dehydrogenase (PDH) in vivo by decreasing phosphorylation of PDH (p-PDH) (Yin et al., J Mol Cell Cardiol 2013). Whilst perhexiline is marketed clinically as a racemic mixture, little is known about the activity of the individual enantiomers. This study aimed to compare the effects of racemic-, (+)- or (-)-perhexiline on the activation of PDH following isoprenaline-induced metabolic stress.

**Methods:** Dark Agouti rats were pre-treated with vehicle or 200 mg/kg/day p.o. of racemic-, (+)- or (-)-perhexiline maleate for 2 weeks before injection with 50 mg/kg i.p. of isoprenaline HCl (or saline). Animals were sacrificed 48 hours later, and total PDH and p-PDH were determined in heart tissue by western blotting, and expressed as normalized arbitrary units. Between-group comparisons were performed by analysis of variance with Tukey’s or Dunnett’s multiple comparison tests.

**Results:** There were no significant differences in total PDH expression, however, isoprenaline-treated animals had significantly higher (p=0.01) mean (SD) p-PDH (3.31 (1.71), n=8) compared to untreated controls (1.40, (0.79), n=7). The effect of isoprenaline was significantly attenuated by pretreatment with either (-)-perhexiline (0.59 (0.67), n=8, p<0.0001) or (+)-perhexiline (1.23 (0.33), n=6, p=0.01), whilst the effect of racemic perhexiline (2.24 (1.20), n=5) was of borderline significance (p=0.07). Compared to isoprenaline alone, only (-)-perhexiline significantly decreased the ratio of p-PDH:total PDH (2.17 (3.24) versus 6.61 (2.05) p<0.01).

**Conclusions:** Our observations are generally consistent with the previously reported effect of perhexiline on PDH activation. However, in the presence of catecholamine-induced stress, they suggest enantioselectivity in the restoration of myocardial PDH activation, in favour of (-)-perhexiline.
### ISCP 23: THE NOVEL PHARMACOLOGICAL TREATMENT OF SYMPTOMATIC POSTPRANDIAL HYPOTENSION BY XANTHINE DERIVATIVES AND PREPARATIONS

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**Background:** Postprandial hypotension (PPH) is an important and frequent problem, particularly in the elderly. Several studies have suggested that caffeine, acarbose, DL-DOPS, Guar gum and octreotide can improve postprandial symptoms and result in reduction in postprandial blood pressure (BP). However, these treatments may not necessarily be effective in the patients with symptomatic PPH.

**Aims:** In the present study, we sought to determine whether Xanthine derivatives and preparations, an adenosine antagonist same as caffeine which block splanchnic methylxantine-sensitive adenosine receptors could be effective for PPH showing resistance to other treatments.

**Methods:** The first case, a 74-year-old man under outpatient care for hypertension was admitted to Aoyama Hospital, Tokyo Women’s Medical University, Tokyo, Japan, for the investigation and treatment of syncope after breakfast in June 2014. Ambulatory BP monitoring (ABPM) over a 24 hours period showed a decrease in systolic BP of more than 20 mmHg within 2 hours after meal, leading to the diagnosis of PPH. We examined meal terrace test (high carbohydrate meal; 60-70g) using ABPM, the patient settled supine position during 2 hours after eating. We investigated and compared the effects of voglibose (0.6mg/day), Guar gum, and theophylline (400mg/day) by ABPM.

We examined the same meal terrace test to the second patient, a 75-year-old man for the treatment of faintness after breakfast in September 2014.

**Results:** In the first patients, having only the meal, BP decreased from 149/63 to 85/46mmHg at 30 minutes after eating. Similar results were observed with administration of voglibose and Guar gum (BP: from 121/79 to 74/51mmHg, from 121/63 to 89/55mmHg, respectively). Surprisingly, theophylline treatment resulted in a smaller reduction in BP at 30 minutes (BP from 124/68 to 117/68mmHg) and within 2 hours after eating (minimum BP 107/71 mmHg).

In the second patients, the same result was obtained. BP was decreased from 121/63mmHg to 90/56mmHg at 60 minutes after having only the meal. However, theophylline treatment resulted in a smaller reduction in BP at 60 minutes (BP from 118/47 to 113/60mmHg) and within 2 hours after eating (minimum BP 103/61 mmHg).

**Conclusions:** This is the first report of improvement of postprandial hypotension by xanthine derivatives and preparations. It is suggested that theophylline may be an alternative effective treatment for postprandial hypotension in the elderly.

### ISCP 24: TIME-DEPENDENT CHANGES IN TWO ATHEROGENIC LIPOPROTEINS AFTER SMOKING CESSATION

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**Introduction:** Smoking cessation is associated with increase in body weight. While long-term (over 4 years) smoking cessation certainly reduces cardiac risk, effects of smoking cessation-associated obesity on cardiovascular risks in an early period (within one year) are largely unknown. Serum alpha1-antitrypsin LDL (AT-LDL) and amyloid A/LDL (SAA-LDL) are oxidatively modified LDL complexes which promote atherosclerosis. We have previously reported that the serum level of the AT-LDL is higher in smokers than in nonsmokers, and that the level decreases at 3 months after smoking cessation. We have also demonstrated that larger weight gain after smoking cessation perturbs such decrease at 3 months after the cessation.

**Objectives:** The present study investigated time-dependent changes in AT-LDL and SAA-LDL after smoking cessation and relationships of these changes with weight gain.

**Methods:** In 17 patients who had continued smoking cessation for one year, we measured serum AT-LDL and SAA-LDL levels by the enzyme-linked immunosorbent assay before smoking cessation, and at 3 months and 1 year after smoking cessation.

**Results:** Body mass index (BMI) significantly increased from baseline (pre-cessation) to 3 months after smoking cessation (p=0.027). Serum AT-LDL and SAA-LDL tended to decrease at 3 months after smoking cessation, while the decrease was insignificant in this number of patients. BMI further increased from 3 months to 1 year after smoking cessation (p=0.036). In contrast, both AT-LDL and SAA-LDL significantly decreased from 3 months to 1 year, and from baseline to 1 year after smoking cessation (AT-LDL : p=0.008, SAA-LDL: p=0.019).

**Conclusion:** BMI and levels of two atherogenic lipoproteins, SAA-LDL and AT-LDL, time-dependently change after smoking cessation. In contrast to insignificant decrease in SAA-LDL and AT-LDL levels at 3 months after smoking cessation, the beneficial effect of non-smoking certainly overcomes potential cardiovascular risks by cessation-associated obesity at one year after the cessation.
ISCP 25: STUDY OF THE ROLE OF IVABRADINE IN ACUTE HEART FAILURE

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Introduction: Ivabradine is a drug which acts by selectively blocking If current in the SA Node. It is approved for use in chronic congestive heart failure. In patients with acute decompensated systolic heart failure, tachycardia could be either a compensatory mechanism or contribute to worsening heart failure. There are situations where using a beta blocker is not an option. The present study was planned to assess the feasibility, safety and efficacy of using Ivabradine in acute heart failure.

Methods: A retrospective analysis of 28 patients of acute heart failure (all due to different spectrum of acute coronary syndrome) in whom ivabradine was used was done. All of them had an ejection fraction of <50%, resting heart rate >70 bpm and SBP> 100 mm Hg without inotropes. The patients were receiving standard guideline directed therapy including beta blockers wherever indicated. Ivabradine was started in a dose of 2.5 mg BD and increased upto 7.5 mg BD in accordance with the patient’s clinical condition. The patient’s clinical parameters were recorded at the time of initiation of Ivabradine and 24-hours and 7 days after initiation of therapy.

Results: A total of 28 patients (mean age 60.3 years, 20 males) constituted the study group. Baseline mean Heart rate (HR) was 96 (70-128) bpm and systolic Blood pressure (SBP) was 110 mm Hg(100-134mm Hg). Patients in NYHA II, III and IV numbered 19, 9 and 0 respectively when the therapy with ivabradine was started. HR decreased by 2.7 ± 0.2 bpm after 24 hours (p =NS) and 14.3 ± 7.2 bpm at day 7 (p = 0.008). The systolic blood pressure decreased by 2.4 mm Hg after 24 hours (p = NS) and 4.1 mm Hg at day 7 (p=0.091) . Patients in NYHA class II and III at 24 hours was 20 and 8 respectively. At 7 days, 8 patients were in NYHA I, 17 in NYHA II and 3 in NYHA III. No worsening of NYHA class was noted in any patient at 7 days.

Conclusion: Initiating Ivabradine in patients of acute heart failure during hospital stay is both safe and effective.

ISCP 26: POOR QUALITY OF LIFE IN PATIENTS WITH MODERATE RENAL DYSFUNCTION AMONG SURVIVORS OF ACUTE CORONARY SYNDROME

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Background and aims: Patients with coronary artery disease (CAD) have lower health-related quality of life (HRQoL) than the general population. Mean EuroQol visual analogue scale (EQ-VAS) score has been reported to be significantly lower at 12 months after myocardial infarction than in the general population. However, the relation between kidney dysfunction and HRQoL in survivors of acute coronary syndrome (ACS) is largely unknown. We aimed to study how kidney dysfunction impact on self-reported HRQoL one year after an ACS. Methods: We used the SWEDEHEART registry to study all patients below the age of 75 years admitted to Swedish coronary care units for an ACS between 2005-10 and who survived at least 1 year (N=50,507). 16,261 patients were followed up 12 months after the initial event, of which 16,005 patients had available creatinine levels to estimate glomerular filtration rate (eGFR) using CKD-EPI formula and to classify them into Chronic Kidney Disease (CKD) stages defined by NKF-KDIGO. HRQoL was assessed using EuroQol health questionnaire assessing five dimensions (mobility, self-care, daily activities, pain/discomfort and anxiety/depression) and visual analogue score (EQ5D and EQ-VAS respectively). EQ5D index score was calculated by combining the five dimensions.

Results: Patients with CKD 1-2 reported an EQ5D index of 0.82±0.23, which was significantly lower in patients with CKD 3 and 4-5 (0.78±0.25 and 0.69±0.29 respectively; p<0.0005); EQ-VAS showed a similar pattern. After adjustment for patient-characteristics (age, gender, diabetes, hypertension, previous congestive heart failure or stroke) and treatment-characteristics (PCI or CABG during hospitalization and drugs at discharge) patients with CKD 3 were more likely to report problems with mobility (OR 1.43, CI 1.26-1.62), self-care (OR 1.35, CI 1.01-1.80), daily activities (OR 1.27-1.67) and mood (OR 1.19, CI 1.06-1.33). Patients with CKD 4-5 were more likely to report problems with mobility, self-care, daily activities and pain/discomfort.

Conclusion: Kidney dysfunction, including moderate kidney failure, has an independent negative impact on health-related quality of life in patients after ACS one year after the initial event.

ISCP 27: MEASURES DERIVED FROM AMBULATORY BLOOD PRESSURE RECORDINGS ARE POOR PREDICTORS OF OBSTRUCTIVE SLEEP APNEA

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Background and aims: Obstructive sleep apnoea (OSA) is characterized by repetitive upper airway obstructions during sleep, intermittent hypoxia, subsequent arousal, and sympathetic activation. OSA contributes to hypertension and cardiovascular risk. We aimed to investigate whether data derived from 24h ambulatory blood pressure recordings (ABMP) can be used to predict the occurrence of OSA.

Methods: Consecutive patients referred to a cardiovascular risk assessment clinic for a 24-h ambulatory blood
pressure measurement (ABPM) were offered to undergo a simultaneous sleep recording, using a validated screening device (SomnocheckMicro; Weinmann). Only patients with antihypertensive drug treatment or ≥130 mmHg systolic and/or ≥80 mmHg diastolic blood pressure by ABPM were included in the analysis. Patient-reported drug intake was registered. eGFR was calculated using the CKD-EPI formula.

**Results:** 86 patients had complete drug reports and performed the sleep and ABPM recordings. Mean age 57 years, BMI 27.9 kg/m², 65% were male. Median 24-h blood pressure was 136/82 mmHg. Only 27% had a well-controlled 24-h blood pressure (<130/80 mmHg). The prevalence of OSA (apnoea-hypopnoea index [AHI] ≥5/h) was 69%, and 33% had an AHI ≥15/h, usually considered an indication for treatment. We found no correlation between AHI and systolic, diastolic, mean or pulse pressure over 24h, or during day or night (patient-recorded bed-time; r²=0.039 and p=0.70 using multiple regression). Non-dipping pattern (26%) did not correlate with AHI (p=0.31). Systolic blood pressure variability did not correlate significantly with AHI (p=0.14). Patients with OSA had lower day-time heart rate (70±8.0 vs 76±8.7 BPM; p=0.003), despite similar use of betablockade (31% vs 37%; n.s.). We found a tendency towards lower AHI in patients (33%) receiving betablockers (p=0.096). Despite a quite normal eGFR (average 93±13 ml/min/m²) we found a significant negative correlation between eGFR and AHI, also after adjusting for age (adjusted r²=0.17; p=0.01).

**Conclusions:** In patients with hypertension, OSA is common, and measures derived from ABPM are poor predictors of OSA occurrence, while reduced eGFR may be of some value.

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**ISCP 28: VASOMOTOR REACTIVITY OF RAT AORTA IN A MODEL OF TAKO-TSUBO CARDIOMYOPATHY**

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**Background & Aims:** Tako-tsubo cardiomyopathy (TTC) is a form of largely reversible dysfunction of regions of the heart, usually precipitated by severe stress. Many animal models of TTC have been developed, but are mainly based on young male rats. In view of the predominant occurrence of TTC in ageing women, we sought to develop an animal model in ageing female rats, with both echocardiographic and immunohistochemical evidence of TTC-like changes in the heart, 24-hours post-treatment with isoprenaline. We now aim to examine the effect of this treatment on vasomotor reactivity of systemic vessels.

**Methods:** Fifteen female Sprague Dawley rats (18-20weeks) were either treated with a single intraperitoneal injection of isoprenaline (5mg/kg; n=9) or acted as controls (n=6). At 24 hours, the aorta was removed, cut into rings and mounted in Krebs solution at 37 degrees Celsius. Viability was assessed with potassium physiological saline solution (KPSS). Norepinephrine (NE) was then applied cumulatively and contractile responses were normalised with respect to the potassium-induced contraction (KPSS). Endothelium dependent relaxation was also assessed via cumulative application of acetylcholine (ACh) in segments pre-constricted with a submaximal concentration of NE.

**Results:** In our aged female rat model, there appears to be no difference in contractile responses to either KPSS (2.33 +/- 0.40g vs 2.13 +/- 0.24g, mean +/- SEM), NE half maximal concentration (EC50, -7.71 +/- 0.21 log M vs -7.67 +/- 0.13 log M) or NE maximal contraction (52.26 +/- 9.86 %KPSS vs 59.05 +/- 7.68 %KPSS) between the control and isoprenaline treated groups respectively. Maximal relaxation to ACh also does not differ between groups (94.68 +/- 1.58 % control vs 91.86 +/- 3.58% isoprenaline-treated) but there is a trend towards reduced potency of ACh in the isoprenaline treated animals (ACh EC50 -7.52 +/- 0.10 log M) compared to the control group (ACh EC50 -7.78 +/- 0.05 log M) (p=0.0597, unpaired t-test), indicating a slight impairment of ACh responses following 24 hours isoprenaline treatment.

**Conclusions:** TTC may produce a small effect on endothelial function in systemic vessels.

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**ISCP 29: AURAPTENE, A COUMARIC COMPOUNDS ANALOGOUS, PREVENTED CARDIAC HYPERTROPHY BY ACTIVATING PPARA AFTER MYOCARDIAL INFARCTION IN RATS**

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**Introduction:** Heart failure is associated with pathological growth and mitochondrial dysfunction of constituent cardiomyocytes. To achieve effective oral pharmacological therapy for heart failure, we screened compounds isolated from natural products and found that aurapten derived from the peel of Citrus Hassaku may be applicable to pharmacological therapy for heart failure.

**Hypothesis:** We assessed the hypothesis that aurapten derived from the peel of Citrus Hassaku may be applicable to pharmacological therapy for heart failure.

**Methods and Results:** One week after operation, 22 rats could a moderate size of MI (Fractional shortening (FS) < 40%) were then randomly assigned to vehicle (n=8), aurapten low-dose (5 mg/kg/day, n=7), or high-dose (50 mg/kg/day, n=7). Oral daily treatments with these agents were continued for 6 weeks. There were no differences in left ventricle (LV) geometric and functional data among the 3 MI groups before treatment. After treatment, LVFS was significantly higher in the aurapten low-dose (21%, p < 0.0001) and high-dose (26%, p < 0.0001) groups than the vehicle group (16%). LV wall thickness in the remote non-infarct area was significantly thinner in the aurapten low-dose (1.4 mm, p < 0.01) and high-dose (1.2 mm, p < 0.0001) groups than the vehicle group (2.5 mm). Histological analysis demonstrated that aurapten...
treatment significantly suppressed MI-induced increases in myocardial cell diameter and perivascular fibrosis compared with vehicle treatment. Moreover, chronic MI induced the activation of ANF, BNP, and MCP1 mRNA levels and the down-regulation of mitochondrial- and lipid metabolism-related gene transcriptions, such as PGC-1α, MFN2, MCAD, mCPT1 in rat LV. Aurapten treatment also improved these gene changes. In cultured cardiomyocytes, aurapten dose-dependently (2.5-10 μM) repressed phenylephrine-induced hypertrophic responses such as increase in cell size and ANF and ET-1 promoter activations and activated mitochondrial- and lipid metabolism-related gene transcriptions. These changes were reversed by MK886, a peroxisome proliferator-activated receptor α (PPARα) antagonist but not GW9662, a PPARγ antagonist.

Conclusions: These results indicated that aurapten prevented cardiac hypertrophy-responses and the worsening of LV systolic function through the activation of PPARα. A natural compound, auraptenene is expected as a novel useful agent for heart failure therapy in humans.

ISCP 30: RECEPTOR FOR ACTIVATED PROTEIN KINASE C1 SUPPRESSES HYPERTROPHIC RESPONSES BY INHIBITION OF THE ASSOCIATION BETWEEN P300 AND GATA4

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Background and Aims: A zinc finger protein GATA4 associates with an intrinsic histone acetyltransferase (HAT), p300 and regulates myocardial gene transcription in response to hypertrophic stimuli. We previously identified a Receptor for Activated Protein Kinase C1 (RACK1), a multi-functional scaffold protein, as a novel GATA4-binding partner and revealed that RACK1 regulates p300/GATA4-dependent transcriptional pathway and hypertrophic responses in cardiomyocytes. However, the molecular mechanism by which RACK1 suppresses p300/GATA4-dependent gene transcription remains unknown. The goal of this study was to clarify how RACK1 works in p300/GATA4 transcription pathway and cardiomyocyte hypertrophy. We assessed the hypothesis that RACK1 reduces the PE-induced increase of the interaction between GATA4 and p300 in cardiomyocytes.

Methods: HEK293T cells and primary cultured cardiomyocytes were harvested for immunoprecipitation followed by western blotting (IP-WB) and chromatin IP (ChIP) assay to examine GATA4 acetylation, DNA binding activity and protein interactions. Cardiomyocytes transduced with or without RACK1 were stained with β-myosin heavy chain (β-MHC) antibody and measured the myocardial cell surface area. Using Dasatinib as a tyrosine kinase inhibitor, we performed IP-WB to examine the phosphorylation of RACK1 in cardiomyocytes.

Results: Overexpression of RACK1 inhibited p300- and phenylephrine (PE)-induced GATA4 acetylation in HEK293T cells and cardiomyocytes, respectively. ChIP assay revealed that RACK1 suppressed p300- and PE-induced DNA binding activities of GATA4. Moreover, RACK1 overexpression significantly inhibited PE-induced hypertrophic responses in cardiomyocytes. RACK1 overexpression decreased the association between p300 and GATA4 in HEK293T cells. Also, RACK1 overexpression inhibited the PE-induced association between p300 and GATA4 in cardiomyocytes. Finally, we examined the phosphorylation of RACK1 and the association between RACK1 and GATA4. PE stimulation increased the tyrosine phosphorylation of RACK1 and decreased the association between RACK1 and GATA4 in cardiomyocytes. This phosphorylation was inhibited by a tyrosine kinase inhibitor, Dasatinib.

Conclusions: These findings demonstrated that RACK1 disrupted p300/GATA4 complex and inhibited hypertrophic responses in cardiomyocytes. Tyrosine phosphorylation of RACK1 may be a trigger of the dissociation of RACK1 from p300/GATA4.

ISCP 31: MANAGEMENT PRACTICES IN HYPERTENSION: RESULTS OF WIN-OVER-A PAN INDIA REGISTRY

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Background: Hypertension is a common disease seen in clinical practice and is associated with high morbidity and mortality. Many patients require combination therapy for the management of hypertension. Objective: To evaluate co-morbidities, risk factors and management practices of hypertension in Indian population. Material and methods: A total of 1596 hypertensive adult patients received anti-hypertensive medications were studied in a cross-sectional, multi-centric, non-interventional, observational registry. Statistical analysis: Categories or nominal data was expressed as numbers with percentages. Continuous variables were analyzed by descriptive statistics using mean, SD, and range Chi square test was used for in between group comparison. Results: The study included 73.50% males and 26.50% females. Overweight (50.50%) and obesity (30.01%) was common in the hypertensive patients (n=903). A total of 54.76% patients had history of smoking. Alcohol use (33.08%), sedentary life style (32.96%) and history of tobacco chewing (17.92%) were the other lifestyle habits of hypertensive patients. Diabetes (36.03%) and dyslipidemia (39.79%) history was common in these patients. Family history of hypertension and diabetes was seen in 82.21% and 45.99% patients respectively. Most (89.16%) patients were treated with combination of antihypertensive agents. ARBs were the by far most commonly used agents (91.98%) followed by calcium channel blockers (68.23%) and diuretics (60.21%). ARB was the most (80.35%) preferred agent as monotherapy. ARB was also the most common agent as a component of dual therapy, four drug and five drug combinations.

Conclusion: Most of the hypertensive agents need combination treatment with antihypertensive agents. ARBs are the most preferred agents as monotherapy for the management of hypertension. ARBs are also very commonly used as a component of combination therapy during hypertension management.

Key words: Antihypertensive, Hypertension, Management
ISP 32: THE DPP-4 INHIBITOR LINAGLIPTIN AND THE GLP-1 RECEPTOR AGONIST EXENDIN-4 PREVENT HIGH GLUCOSE-INDUCED IMPAIRMENT OF ENDOTHELIAL FUNCTION IN RAT MESENTERIC ARTERIES

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Background and Aims: Hyperglycaemia in diabetes increases oxidant stress leading to impaired endothelial function. We hypothesized that DPP-4 inhibitors (linagliptin, sitagliptin, vildagliptin) and the GLP-1 receptor agonist exendin-4 (Ex-4) would improve relaxation in mesenteric arteries in the presence of high glucose.

Methods: Endothelium-dependent and –independent relaxation to acetylcholine (ACh) and sodium nitroprusside (SNP) was determined in mesenteric arteries from Wistar rats pre-contracted with phenylephrine and exposed for 2 hours to normal (11 mM) or high (40 mM) glucose concentrations.

Results: Incubation of mesenteric arteries in 40 mM glucose caused a significant impairment of endothelium-dependent relaxation (ACh pEC50 11 mM 7.31±0.07 vs 40 mM 6.32±0.2 p<0.05), but did not affect responses to SNP. In addition, 40 mM glucose caused a significant increase in superoxide levels in mesenteric arteries assayed by L-012 (11 mM, 2384±210; 40 mM, 4458±420 counts/mg, n=6, p<0.0001). Superoxide levels were significantly reduced in the presence of linagliptin (1101±172 counts/mg, n=7, p<0.001) and Ex-4 (3064±250 counts/mg, n=7, p<0.0001) but not by the other DPP-4 inhibitors. Co-incubation with linagliptin (1μM) or Ex-4 (1μM) (ACh pEC50 glucose 40 mM+linagliptin=7.25±0.06, glucose 40 mM+ Ex-4=6.80±0.05 p<0.05) prevented impairment of endothelium-dependent relaxation caused by high glucose but sitagliptin and vildagliptin had no effect. The presence of the GLP-1 antagonist exendin-fragment (9-39) prevented the beneficial effect of Ex-4 but did not alter the improvement of endothelium-dependent relaxation by linagliptin. When the contribution of NO was abolished by N-nitro-L-arginine (100 μM) plus a soluble guanylate cyclase inhibitor (ODQ, 10 μM), or the contribution of endothelium-dependent hyperpolarisation (EDH) was inhibited with TRAM-34 (1μM) plus apamin (1μM), ACh-induced relaxation was significantly impaired by high glucose indicating impaired the contributions of both NO and EDH. The DPP-4 inhibitor linagliptin significantly improved ACh-induced relaxation in the presence of either group of inhibitors.

Conclusions: The DPP-4 inhibitor linagliptin and the GLP-1 receptor agonist exendin-4 have antioxidant effects that are not shared by sitagliptin and vildagliptin. Further, unlike Ex-4, the beneficial actions of linagliptin do not involve an action of the GLP-1 receptor. Linagliptin and Ex-4 may preserve the contribution of both NO and EDH independently of any glucose lowering activity to improve vascular function in diabetes.

ISP 33: EFFECTS OF BLOOD PRESSURE LOWERING ON CARDIOVASCULAR RISK ACCORDING TO BASELINE BODY MASS INDEX: A META-ANALYSIS OF RANDOMISED TRIALS

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Background: Recent reports have suggested that the benefits of blood pressure lowering in obese people compared to people of normal weight may depend upon the choice of drug. We sought to confirm or refute these findings from individual studies by doing a meta-analysis based upon individual patient data using multiple trials included in the Blood Pressure Lowering Treatment Trials’ Collaboration.

Methods: We compared the effects of blood pressure lowering regimens based upon different drug classes for the primary outcome of total major cardiovascular events. Meta-analyses and meta-regressions were used to seek evidence for interactions between treatment and body mass index when fitted as either a categorical (<25, 25-30, >30kg/m2) or a continuous variable.

Results: Analyses were done on 135,715 individuals drawn from 22 trials who experienced a total of 14,353 events. For none of the six primary comparisons made was there evidence that protection varied by drug class across the three BMI groups studied (all p for trend >0.20). When analysed as a continuous variable there was a slightly greater protection for each 5kg/m2 higher BMI when using an ACE inhibitor compared to a calcium antagonist (7%, 95% confidence interval 2-11%, p=0.004) or a diuretic (7%, 2-11%, p=0.002). The meta-regressions identified no relationship between BMI category and the risk reduction achieved for a given fall in systolic blood pressure. In contrast to a major prior report, we found no relationship between BMI and the efficacy of calcium antagonists compared to diuretics.

Conclusion: These analyses provide little evidence that the selection of a particular class of blood pressure lowering drug will substantially alter outcomes for individuals that are obese compared to lean.

ISP 34: THE ACTUAL CLINICAL PRAXIS OF INR CONTROL FOR STROKE PREVENTION IN NON-VALVULAR ATRIAL FIBRILLATION

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Objectives: To analyze how precise is INR control in patients of an urban area with previous stroke and atrial fibrillation treated with dicumarine derivatives since in this very high-risk population it is mandatory prevent recurrence and to avoid bleeding.
Methods and results: We analyzed 4590 INR level determinations of 131 patients (age 77±9 years, 63% male, 80% with arterial hypertension, 37% diabetic, 45% with dyslipemia) all with previous stroke and with atrial fibrillation during a follow-up of 25±21 months. The mean number of INR determinations per person was 35±26. A total of 2013 INR determinations (44%) showed an inappropriate level (<2 or >3). Thus, a mean of 15±11 determinations/person demonstrated a result not in therapeutic range and overall patients were during 8.2±8 months at risk of thromboembolic or bleeding events in relationship to INR out of therapeutic range. During follow-up, cardiovascular events (any ischemic or hemorrhagic) occurred in 52% (n=68) of patients. INR determinations obtained at time of cardiovascular events (n=54) showed an inappropriate level of anticoagulation in 38 patients (70%). Furthermore, cardiovascular and total mortality were of 26% (n=35) and 43% (n=57), respectively. A total 88 patients (67%) suffered any cardiovascular event or died during the follow-up period.

Conclusions: Appropriate INR control is mandatory for prevention of serious cardiovascular complications, especially in patients with previous stroke and atrial fibrillation. This population shows a very high risk of suffering cardiovascular events and mortality during the follow-up. In clinical practice, management of oral anticoagulation with dicumarine derivates is far of being optimal, even in urban areas.

ISCP 35: ARE LIPID LEVELS AFTER PERCUTANEOUS CORONARY INTERVENTION APPROPRIATE?


Univ. Hospital of Sabadell, (Univ Autònoma of Barcelona), Department of Cardiology, Sabadell, Spain

Objectives: To assess the control of total cholesterol (TC), LDL-C, HDL-C, and triglycerides (TG) in patients undergoing percutaneous coronary intervention (PCI) and if treatment changes during follow-up were appropriate or not according to the values of LDL achieved.

Methods and results: we analyzed data of 307 consecutive patients undergoing PCI from Jan-2007 to Sep-2009. During follow-up we evaluated all available lipid plasma levels and the incidence of cardiovascular (CV) events (myocardial infarction, stroke, readmission for ACS, or new revascularization procedure), CV mortality, and overall mortality. We also tested if the treatment strategy was appropriate or not, according to the LDL-C value achieved in every control with two target levels (70 and 100 mg/dL). Mean follow-up was 44±16 months. After PCI 293/307 patients (95.4%) were treated with statins. Before PCI, the mean values were: TC 195±41 mg/dL, LDL-C 115±348 mg/dL, HDL-C 48±14 mg/dL, and TG 155±96 mg/dL. At the end of follow-up they were: CT 163±38 mg/dL, LDL-C 89±31 mg/dL, HDL-C 46±12 mg/dL and TG 145±99 mg/dL. In 46 patients there was not any LDL-C determination after PCI (20 deaths, and 26 lost of follow-up). We analyzed 1.087 determinations of LDL-C from 261 patients. Considering a target level of LDL-C <70 mg/dl and of <100 mg/dl, this was only achieved in 28.97% and in 72.4% of cases, respectively. We analyzed the therapeutic strategy adopted by the physician in 1.008 cases. Considering the target levels of LDL-C of <70 mg/dl and of <100 mg/dl, the change in the therapeutic strategy was appropriate only in 34.92% and 72.4% of cases, respectively.

Conclusions: After PCI, despite a high rate of statin therapy and a significant reduction in mean values of lipid levels, a high proportion of patients did not achieve the target level of LDL-C. Thus, in usual practice, guidelines are not followed properly.

ISCP 36: LIPID CONTROL IN DIABETIC VERSUS NON-DIABETIC PATIENTS AFTER PERCUTANEOUS CORONARY INTERVENTION.


Univ. Hospital of Sabadell, (Univ Autònoma of Barcelona), Department of Cardiology, Sabadell, Spain

Objectives: To assess the control of total cholesterol (C), LDL-C, HDL-C, and triglycerides (TG) in patients (P) undergoing percutaneous coronary intervention (PCI) and if treatment changes during follow-up were appropriate or not according to the values of LDL achieved.

Methods and results: We analyzed lipid level determinations of 131 patients (age 77±9 years, 63% male, 80% with arterial hypertension, 37% diabetic, 45% with dyslipemia) all with previous stroke and with atrial fibrillation during a follow-up of 25±21 months. The mean number of INR determinations per person was 35±26. A total of 2013 INR determinations (44%) showed an inappropriate level (<2 or >3). Thus, a mean of 15±11 determinations/person demonstrated a result not in therapeutic range and overall patients were during 8.2±8 months at risk of thromboembolic or bleeding events in relationship to INR out of therapeutic range. During follow-up, cardiovascular events (any ischemic or hemorrhagic) occurred in 52% (n=68) of patients. INR determinations obtained at time of cardiovascular events (n=54) showed an inappropriate level of anticoagulation in 38 patients (70%). Furthermore, cardiovascular and total mortality were of 26% (n=35) and 43% (n=57), respectively. A total 88 patients (67%) suffered any cardiovascular event or died during the follow-up period.

Conclusions: Appropriate INR control is mandatory for prevention of serious cardiovascular complications, especially in patients with previous stroke and atrial fibrillation. This population shows a very high risk of suffering cardiovascular events and mortality during the follow-up. In clinical practice, management of oral anticoagulation with dicumarine derivates is far of being optimal, even in urban areas.

Methods and results: We analyzed data of 307 consecutive patients undergoing PCI from Jan-2007 to Sep-2009. During follow-up we evaluated all available lipid plasma levels and the incidence of cardiovascular (CV) events (myocardial infarction, stroke, readmission for ACS, or new revascularization procedure), CV mortality, and overall mortality. We also tested if the treatment strategy was appropriate or not, according to the LDL-C value achieved in every control with two target levels (70 and 100 mg/dL). Mean follow-up was 44±16 months. After PCI 293/307 patients (95.4%) were treated with statins. Before PCI, the mean values were: TC 195±41 mg/dL, LDL-C 115±348 mg/dL, HDL-C 48±14 mg/dL, and TG 155±96 mg/dL. At the end of follow-up they were: CT 163±38 mg/dL, LDL-C 89±31 mg/dL, HDL-C 46±12 mg/dL and TG 145±99 mg/dL. In 46 patients there was not any LDL-C determination after PCI (20 deaths, and 26 lost of follow-up). We analyzed 1.087 determinations of LDL-C from 261 patients. Considering a target level of LDL-C <70 mg/dl and of <100 mg/dl, this was only achieved in 28.97% and in 72.4% of cases, respectively. We analyzed the therapeutic strategy adopted by the physician in 1.008 cases. Considering the target levels of LDL-C of <70 mg/dl and of <100 mg/dl, the change in the therapeutic strategy was appropriate only in 34.92% and 72.4% of cases, respectively.

Conclusions: After PCI, despite a high rate of statin therapy and a significant reduction in mean values of lipid levels, a high proportion of patients did not achieve the target level of LDL-C. Thus, in usual practice, guidelines are not followed properly.
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