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Project title: Targeting activated platelets as early makers for diagnosis and treatment of inflammatory diseases.

Laboratory: Atherothrombosis and Vascular Biology, Baker IDI Heart & Diabetes Institute

Primary Supervisor(s): Dr Bock Lim & Prof Karlheinz Peter

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Research Focus: Using a unique single-chain protein that targets activated platelets, this project will examine the role of platelets during the development of various inflammatory conditions with the aim of generating novel diagnostic tools and drugs for clinical purposes.

Keywords: Nanoparticles, imaging, Sortase bio-enzymatic conjugation, thrombosis, inflammation

Project description:
Besides their critical role in haemostasis, platelets are recently associated with immune defense and inflammation. Activated platelets are shown to play a critical role in various inflammatory diseases such as atherosclerosis, pulmonary embolism, rheumatoid arthritis and multiple sclerosis. Hence early detection of these cells during inflammatory progression (diagnosis) and effective inhibition of their function (therapy) are both critical in the clinical combat against these conditions.

We have generated a single chain antibody which binds to activated platelets. When linked with a suitable detector, such as a near-infra red fluorescent probe, this single-chain can be used as diagnostic marker. The linked protein can be used to detect the presence of activated platelets in areas of the body where inflammation associating with the disease is occurring by using a highly specialized, novel and world’s first Fluorescence Emission Computed Tomography scanner (FLECT). The Figure shows the detection of fluorescence signal by the scanner in the liver of a mouse.

In addition, when linked to a specific drug, this single chain antibody can direct treatment to the site of inflammation where activate platelets are and thereby reduce drug dosage and undesirable side-effects.

This project will examine the diagnostic and therapeutic capabilities of this single chain antibody in the above diseases using different mouse models.

The student will learn molecular biology, recombinant protein production and purification, modern drug delivery methods as well as most advanced imaging technologies.

Project related methods/skills/technologies:
- Cloning, protein expression and purification
- Flow-cytometry and fluorescent microscopy
- Fluorescence Emission Computed Tomography (FLECT) scanning
- Mouse disease models
- Modern drug delivery
- Advanced imaging technologies

References:
**Project title:** Identification of new treatments for unstable atherosclerosis plaque in a novel mouse model

**Laboratory:** Atherothrombosis and Vascular Biology

**Primary Supervisor(s):** Dr Yung Chih (Ben) Chen, Prof Karlheinz Peter

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**Research Focus:** Unstable plaque/ Coronary artery disease/microRNA therapeutics

**Keywords:** Coronary artery disease; unstable plaque; mouse model; microRNA

**Project description:**
Atherosclerotic plaque rupture and its complications such as myocardial infarction is one of the major causes of morbidity/mortality worldwide. Currently, research towards detecting plaques that are prone to rupture and towards preventing rupture is limited by the lack of animal models that reflect atherosclerotic plaque morphology as seen in humans.

We developed a novel mouse model that truly reflects human plaque rupture (1). Several genes and microRNAs have been found to be associated with plaque rupture in our model. We would like to identify genes and microRNAs that can be used/targeted for therapeutic strategies with the ultimate goal to prevent plaque rupture and thus myocardial infarctions.

This project will be focused on the development of a new type of therapy, microRNA therapeutics. It will establish their use in cell culture and in animal experiments.

**Project related methods/skills/technologies:**
- microRNA therapeutics
- molecular biology
- cell culture; RT-PCR; Western blot
- small animal surgery
- histology
- immunohistochemistry
- confocal microscopy
- intravital microscopy
- flow cytometry

**References:**
Project title: *Molecular ultrasound imaging of thrombotic diseases using novel platelet-targeted microbubbles*

**Laboratory:** Atherothrombosis and Vascular Biology

**Primary Supervisor (s):** Dr Xiaowei Wang, Prof Karlheinz Peter

**Contact:** karlheinz.peter@bakeridi.edu.au; Xiaowei.wang@bakeridi.edu.au; 85321490; 85321495

**Research Focus:**
Despite primary and secondary prevention, thrombotic and embolic events such as myocardial infarction and stroke remain a major health issue and are leading causes of mortality and morbidity in Australia and worldwide. Research in the Atherothrombosis and Vascular Biology lab focuses on translational research that links the findings from basic science to the practical applications that enhance human health and well-being in the clinical settings. Molecular ultrasound imaging of thrombi/emboli would allow for early diagnosis and therefore timely and appropriate medical intervention.

**Keywords:** Cardiovascular, Molecular Imaging, Ultrasound, Translational Research

**Project description:**

Our lab has extensive experience with small recombinant antibodies that bind to the activated platelets on thrombi\(^1\). When these antibodies are coupled to contrast agents, we could use them for diagnostic imaging and monitoring of treatment\(^2,3\). In addition, these antibodies could be coupled to thrombolytic drugs and be used for targeted therapy, thereby avoiding the bleeding complications\(^3,4\).

We have showed that these antibodies, when conjugated to microbubbles, could be used for molecular ultrasound imaging of thrombotic diseases\(^2,3\). The *Figure shows the increase binding of targeted microbubbles (LIBS-MB) to the thrombus in the carotid artery of mice.*

Our current aim to use this technology to image several other thrombotic disease models, such as venous thrombosis, atrial fibrillation, atherosclerosis (plaque instability) and myocardial infarction.

**Significance:** With steadily increasing health care expenses, a promising translational imaging application using ultrasound can fulfil the need for a cost-effective and non-invasive diagnostic tool, thereby reducing the mortality and morbidity of cardiovascular diseases.

**Project related methods/skills/technologies:**
- Production and purification of recombinant proteins for diagnosis and therapy
- SDS PAGE and Western blots
- Flow cytometry
- Generation of functionalized imaging contrast agents
- Ultrasound imaging
- Immunohistochemistry

**References:**
Project title: Diagnosis and therapy of inflammatory diseases using molecular ultrasound imaging

Laboratory: Atherothrombosis and Vascular Biology

Primary Supervisor (s): Dr Xiaowei Wang, Prof Karlheinz Peter

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Research Focus:
Research in the Atherothrombosis and Vascular Biology lab focus on translational research that links the findings from basic science to the practical applications that enhance human health and well-being in the clinical settings. Molecular ultrasound imaging would allow for early diagnosis and therefore timely and appropriate medical intervention.

Keywords: Inflammation, Molecular Imaging, Ultrasound, Translational Research

Project description:
The use of small recombinant antibodies for diagnostic molecular ultrasound imaging and targeted drug delivery is well established in our lab1-4. Ultrasound imaging offers significant advantages: It is already a well established clinical imaging technique and the equipment required is already available in most hospitals. It is non-invasive, cost effective, real-time and does not involve ionising irradiation. There is no known long-term side effect of ultrasound imaging. Ultrasound imaging is well suited for routine clinical application where frequent imaging is needed, such as broad screening programs for early disease detection. In addition, contrast enhanced ultrasound with bubbles has been successfully introduced into the clinic and there is a high probability that our targeted microbubble approach can be rapidly translated into clinical practice.

This project would focus on imaging of inflammatory conditions. Peritonitis is the inflammation of the abdominal cavity and a serious clinical complication after the rupture of organs upon abdominal trauma or appendicitis. Optimised early therapy of these conditions is hindered by the lack of fast, real-time imaging technologies. Molecular imaging has the potential to provide early diagnosis thereby allowing timely therapy and prevention of sepsis.

One of the endothelial surface molecules most strongly and specifically upregulated in inflammation is the Vascular Cell Adhesion Molecule-1 (VCAM-1). For this reason this molecule has been chosen as an additional target epitope for molecular imaging of inflammation. We propose to conjugate VCAM-1 targeting recombinant antibodies to ultrasound contrast agents for imaging via contrast enhanced ultrasound. We would use this recombinant antibody for diagnosis imaging and targeted delivery of pharmacological treatment.

Significance: With steadily increasing health care expenses, a promising translational imaging application using ultrasound can fulfil the need for a cost-effective and non-invasive diagnostic tool.

Project related methods/skills/technologies:
- Production if recombinant proteins for diagnosis and therapy
- SDS PAGE and Western blots
- Flow cytometry
- Generation of functionalized imaging contrast agents
- Ultrasound imaging
- Immunohistochemistry

References:
Project title: Use of vessels-on-a-chip to develop anti-thrombotic nanoparticles

Laboratory: Atherosclerosis and Vascular Biology Laboratory, Baker IDI Heart and Diabetes Institute

Primary Supervisor(s): Dr Erik Westein, Prof Karlheinz Peter

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Research Focus:

Keywords: Atherosclerosis, thrombosis and blood shear stress

Project Description:

Anti-platelet therapy, used to treat atherothrombosis, suffers from substantial bleeding complications because it is not tailored to act exclusively at sites of pathological thrombus formation where blood shear stresses are typically very high. In this project we will investigate mechanisms of thrombus formation that predominate at high shear stress conditions and provide a microfluidic approach in which phospholipid based liposomes will be used to deliver high levels of anti-platelet drugs specifically at sites of high shear stress. We recently developed relevant models of thrombosis and atherosclerosis, both in vivo and in microfluidic flow devices allowing us to develop and characterize drug loaded liposomes. This study is a step towards safer and more potent inhibition of thrombus formation with anti-platelet drugs while minimizing systemic bleeding complications. This diverse project will combine platelet biology, microfluidic technology and high-end microscopy to adequately address the research questions.

Project related methods/skills/technologies:

- Microfluidics
- Live microscopy (Fluorescence)
- Isolation of blood platelets
- Flow cytometry
- Animal thrombosis models

References:

**Project title:** Vessels-on-a-chip to study blood flow dependent thrombotic processes

**Laboratory:** Atherosclerosis and Vascular Biology Laboratory, Baker IDI Heart and Diabetes Institute

**Primary Supervisor(s):** Dr Erik Westein, Prof Karlheinz Peter

**Contact:** Erik.westein@bakeridi.edu.au 8532 1479

**Research Focus:**

Keywords: Atherosclerosis, thrombosis and blood shear stress

**Project description:**

This project focuses on the processes that modulate arterial thrombosis, with a particular emphasis on the contribution of blood flow dynamics. We have previously demonstrated that thrombi themselves create flow conditions that promote further thrombus growth. Also, the presence of atherosclerotic plaques that have grown intraluminal are pro-thrombotic due to their geometries. A key player in the pro-thrombotic state of the blood in areas of altered flow dynamics is the blood-borne protein von Willebrand Factor (vWF). This multimeric plasma protein is critical in platelet aggregation under arterial blood flow conditions and becomes “activated” at sites of severe vessel narrowing. Aortic valve stenosis is such a medical condition where the aortic valve is calcified, leading to a reduced lumen during systolic blood flow.

The aim of this project is to delineate the pro-thrombotic effects of aortic valve stenosis and its effect on vWF. We have developed a range of microfluidic vessels, or vessels-on-a-chip, to study the effects of blood flow on the thrombotic process. The microfluidics will emulate geometries of aortic valves with various degrees of stenosis. This diverse project will combine platelet biology, microfluidic technology and high-end microscopy to adequately address the research questions.

**Project related methods/skills/technologies:**

- Microfluidics
- Live microscopy (Fluorescence)
- Isolation of blood platelets
- Flow cytometry
- Animal thrombosis models

**References:**

Project title: Targeted tannic acid nanocapsules for molecular imaging and drug delivery

**Laboratory:** Vascular Biotechnology

**Primary Supervisor(s)**
Prof Christoph Hagemeyer (Baker IDI) / Prof Frank Caruso (University of Melbourne)

**Contact:**
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**Research Focus:**
Recombinant antibodies, imaging, nanotechnology, bioconjugation, targeted therapy

**Keywords**
Nanoparticles, imaging, Sortase bio enzymatic conjugation, thrombosis, inflammation

**Project description:**
The aim of this project is to generate antibody targeted nanocapsules made out of the natural polyphenol tannic acid and metal ions (Ref 1) as imaging agents and drug delivery vehicles for the treatment and diagnosis of cardiovascular disease. The ease, low cost, and scalability of the capsule assembly process, combined with high biocompatibility, makes tannic acid a very interesting nanomaterial for biomedical applications.

This project is furthermore based on the single-chain antibody (scFvs) technology, an emerging class of biotechnologically produced therapeutics (Ref 2). ScFvs are designed to contain the variable regions of a full IgG antibody linked by a small linker. The sequence of scFvs can be encoded on a single plasmid and thus mutations and tags can be added using molecular biology techniques.

We have generated specific single-chain antibodies (scFv) that can selectively target markers associated with atherosclerosis, thrombosis cancer. These include 1) MAN-1, which targets the activated form of the Mac-1 integrin on monocytes and macrophages and thus is specific for these leukocytes in the activated state; 2) scFv59D8, which targets fibrin, the end product of humoral coagulation; 3) scFvanti-LIBS, which targets the platelet surface glycoprotein integrin receptor IIb/IIIa in its activated, ligand bound form and 4) scFvanti-HER, which binds to the HER2 receptor on cancer cells.

These single-chain antibodies will be produced and conjugated to tannic acid nanocapsules using a recently developed bio-click method (Ref 3,4). The antigen-specific binding capacity of the scFv-nanocapsule constructs will be confirmed using fluorescence-labeled particles in flow cytometry. Optimal load and release kinetics will be determined in established in vitro assays. Finally, the novel targeted particles will be investigated in molecular imaging (PET, MRI, Fluorescence) as well as drug delivery applications in relevant animal models (e.g. novel plaque rupture model Ref 5).

The versatile nature of the system and high biocompatibility of all components could have a major impact on the fields of nanotechnology and translational medicine.

**Project related methods/skills/technologies:**
- Protein production and characterisation
- Bioconjugation to nanomaterials
- Molecular imaging

**References:**
**Project title: Recombinant agents and nanoparticles for efficient and safe thrombolysis**

**Laboratory: Vascular Biotechnology**

**Primary Supervisor (s) Dr Thomas Bonnard, Prof Christoph Hagemeyer**

**Contact:** christoph.hagemeyer@bakeridi.edu.au 8532 1494

**Research Focus:**
Recombinant antibodies, nanotechnology, targeted therapy, safer treatments for heart attack and stroke

**Keywords**
Antibodies, thrombolysis, targeting, nanocapsules, bioconjugation, click chemistry

**Project description:**
This project is based on the single-chain antibody (scFv) technology, an emerging class of biotechnologically produced therapeutics. ScFvs are designed to contain the variable regions of a full IgG antibody linked by a small peptide. The sequence of scFvs can be encoded on a single plasmid and thus mutations and additional fusions of tags or effector molecules can be performed using molecular biology techniques (Ref 1).

Our aim is the development of novel targeted thrombolytic drugs to treat thrombosis. We have previously focused on targeting fibrin within blood clots and already showed the benefits of our targeted delivery approach (Ref 2+3). Now we focus on activated GPIIb/IIIa, the platelet receptor for fibrinogen. Its activation represents the final step common to all types of platelet activations. As this receptor’s default state is a non-activated, resting state, GPIIb/IIIa needs to become activated in order to bind its major ligand, soluble fibrinogen causing platelet aggregation and thrombus formation (Ref 4+5). The anti-GPIIb/IIIa scFvs will be fused to a thrombolytic urokinase type plasminogen-activator and a third anticoagulation component (Factor Xa blocker TAP), generating a new class of drugs with thrombolytic and anti-thrombotic properties in a single molecule. TAP is a highly potent and selective factor Xa inhibitor allowing effective anticoagulation because of its central, up-stream, and rate-determining position in the coagulation cascade. Targeting of TAP to clots can decrease systemic anticoagulation and thus bleeding complications. We will also develop targeted nanoparticles filled with thrombolytic drug which specifically release the payload in the presence of pro thrombotic molecules such as thrombin.

By accumulating the fibrinolytic drug at the site of the clot, our designed constructs have the potential to improve the efficacy and safety of thrombolytic treatment by reducing the overall blood concentration and bleeding complications associated with current drugs (Ref 6). We expect a significant faster reopening of the occluded vessel in animal models compared to non-targeted plasminogen activators.

**Project related methods/skills/technologies:**
- Recombinant antibody production and characterisation
- Animal work
- Enzymatic assays
- Nanomaterial generation
- Bioconjugation

**References:**
Project title: Single-chain antibody-targeted nanoparticles for diagnosis of vascular diseases

Laboratory: Vascular Biotechnology

Primary Supervisor (s) Dr Karen Alt, Prof Christoph Hagemeyer

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Research Focus:
Molecular imaging, personalised medicine, biotechnology, better diagnostic tools for clinical use

Keywords
Antibodies, thrombosis, inflammation, targeting, magnetic resonance imaging, bioconjugation, gadolinium

Project description:
This project aims to develop nanoparticle contrast agents for magnetic resonance imaging (MRI) that selectively target molecular markers of cardiovascular disease (CVD). These imaging agents will be developed for the early detection of unstable, rupture-prone, vulnerable atherosclerotic lesions, thrombosis and difficult to diagnose vessel occlusions, such as pulmonary embolism. We have generated specific single-chain antibodies (scFv) that can selectively target markers associated with atherosclerosis and thrombosis (Ref 1). These include 1) MAN-1, which targets the activated form of the Mac-1 integrin on monocytes and macrophages and thus is specific for these leukocytes in the activated state; 2) scFv59D8, which targets fibrin, the end product of humoral coagulation; 3) scFvanti-LIBS, which targets the platelet surface glycoprotein integrin receptor llb/llla in its activated, ligand bound form (Ref 2); and 4) P-selectin, which binds to activated platelets and activated endothelial cells.

We have already demonstrated that coupling of superparamagnetic iron oxide (SPIO)-beads to scFvanti-LIBS and scFvanti P-Selectin can target and image activated platelets in vessels in vitro and in vivo by MRI (Ref 3+4).

To increase sensitivity and avoid the black contrast of SPIOs, we will use Gadolinium (Gd)-loaded dendrimers to give contrast in MRI. Dendrimers have a highly branched, three-dimensional, nanoscale architecture, low polydispersity and multivalent surfaces that allow simultaneous binding of Gd for imaging and scFvs for targeting. To combine the scFv and contrast agent we will employ a novel, highly biocompatible bioconjugation method (Sortase Bio Click) developed in our lab (Ref 5+6).

The antigen-specific binding capacity of the scFv- dendrimer constructs will be confirmed using fluorescence-labelled dendrimers in flow cytometry. Optimal size and Gd-loading will be determined via in vitro MRI of human thrombi. Finally, efficacy studies in a mouse model of atherosclerosis and pulmonary embolism will provide in vivo proof of the imaging capability of the constructs.

Project related methods/skills/technologies:
• Recombinant antibody production and characterisation
• Animal work
• Nanomaterial generation
• Bioconjugation
• Molecular imaging

References:
# Project title: Targeted virus particles to inhibit atherosclerosis

## Laboratory:
Vascular Biotechnology

### Primary Supervisor(s)
Prof Christoph Hagemeyer, Dr Paul Gregorevic (collaborator)

### Contact:
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## Research Focus:
Gene therapy, surface receptor inhibition, targeted therapy, treatment for heart attack and stroke

## Keywords
Antibodies, atherosclerosis, VCAM-1, sortase, molecular biology, adeno-associated virus

## Project description:
The adhesion of leukocytes to endothelium plays a central role in the development of atherosclerosis and thus represents an attractive therapeutic target for anti-atherosclerotic therapies. Single-chain antibodies against epitopes associated with vascular inflammation/dysfunction provide a unique tool for specific targeting of virus particles with inhibitory fusion proteins to areas of need (Ref 1).

This specific targeting provides a new therapeutic approach for atherosclerotic disease. We could show that interference with the cytoskeletal anchorage of adhesion molecules through co-expression of inert fusion proteins, can reduce cell-cell interactions, leading to the disruption of monocyte adhesion to inflamed endothelial cells (Ref 2). We achieved this with a new inhibitory fusion protein containing the intracellular part of vascular cell adhesion molecule-1 (VCAM-1) and the extracellular and transmembrane part of CD7 as an inert marker. We will use adeno-associated virus therapy, a clinically highly relevant system for gene delivery and therapy (Ref 3+4). For the attachment of the antibody to the virus surface we will employ a novel, highly biocompatible bioconjugation method (Sortase Bio Click) developed in our lab (Ref 5+6).

This project aims to develop novel biotechnological approaches that are based on our expertise in single-chain antibodies generation, modification and fusion to effector molecules and nanoparticles. The biological effects of this novel virus targeting approach will be evaluated in a mouse model of advanced atherosclerosis.

A successful outcome of this study will be highly significant. We would have demonstrated the ability of an inhibitory CD7-VCAM-1 construct to block cell adhesion in vivo to reduce atherosclerosis. In addition, we would have developed several methods for single-chain targeted delivery of virus particles that might be generally applicable in basic research and human diseases treatment.

## Project related methods/skills/technologies:
- Recombinant antibody production and characterisation
- Animal work
- Cell biology
- Virus biology
- Modern bioconjugation methods
- Molecular biology

## References:
Project title: Evaluation of regional dysfunction of the aged heart post ischemia/reperfusion injury

Laboratory: Experimental Cardiology

Primary Supervisor(s): Xiao-Jun Du, Helen Kiriazis

Contact: Xiao-Jun Du
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Research Focus: Ageing heart shows increased sensitivity to ischemic injury and aberrant post-injury healing. This project will assess the degree of impact of ageing on post-ischemic cardiac regional dysfunction determined by high-resolution echocardiography.

Keywords
Cardiac regional and global function, echocardiography, ischemia/reperfusion injury, ageing

Project description:
The prevalence of ischemic heart disease increases with age. Moreover, there is a loss of cardioprotection and reduced tolerance to ischemic injury with ageing, leading to more severe cardiac dysfunction. Age-dependent accumulation of collagen in the heart leads to progressive increase in ventricular stiffness and impaired function. Understanding the functional impact of fibrosis in the ageing heart following cardiac ischemia is critical in order to design new therapies. This project will use sophisticated echocardiography (echo) techniques and VevoStrain software, to examine the effects of ischemic duration on regional cardiac dysfunction following ischemia/reperfusion injury in young and aged mice.

Blocked coronary vessels lead to myocardial infarction. In the clinic, these vessels are unblocked allowing reperfusion of the ischemic-damaged heart muscle. We can mimic this condition in mice by inducing ischemia/reperfusion injury. Briefly, the left coronary artery will be tied inducing an ischemic area involving ~40% of the left ventricle (LV). At specific time-points, this occlusion of the artery will be removed to re-establish blood flow, a process called reperfusion. Mice at 3 (young) and 10-12 (aged) months of age will be studied with 3 ischemic durations tested (60, 120 and 240 min). Echocardiography and VevoStrain analysis will be performed at 0 (baseline), 1, 2, 3 and 4 weeks post surgery to assess changes in regional LV wall motion. Echo findings will be related to cardiac histological examination.

The top echocardiography (echo) image shows tracking of two points on the wall of the LV of a normal mouse (arrows point to a blue and red dot on the wall), with the corresponding blue and red velocity traces of these points against time. In a normal heart, movement of different parts of the wall are synchronised, as shown here.

The bottom panel shows a mouse heart after myocardial infarction (MI). It is clear that the wall motion is not synchronised in this animal. In fact, the red line is flat indicating that this part of the heart wall has been severely damaged by MI and is hardly moving.

[Images from FUJIFILM VisualSonics, Inc.]
**Project related methods/skills/technologies:**
- echocardiography
- speckle-tracking based strain analysis
- quantitative histology
- RT-PCR for gene expression

**References:**
Project title: Antioxidant therapy of fibrotic cardiomyopathy to limit arrhythmias

Laboratory: Experimental Cardiology

Primary Supervisor(s): A/Prof Xiao-Jun Du, Dr Helen Kiriazis, My-Nhan Nguyen

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Research Focus: Cardiac fibrosis (scar tissue accumulation) is a major factor for arrhythmias. We will study the possibility that antioxidant therapy is anti-arrhythmic, due largely to its anti-fibrosis effect. This study will be conducted in a transgenic mouse model of fibrotic cardiomyopathy and arrhythmias.

Keywords: cardiomyopathy, myocardial fibrosis, arrhythmias, antioxidant

Project description:

Heart failure is the leading cause of cardiac fatality and a major challenge to modern cardiology. Clinical studies show that about 50% of deaths of heart failure patients are due to arrhythmic sudden death [1]. A failing heart shows significant histopathology including fibrosis, cardiomyocyte hypertrophy and cardiomyocyte apoptosis. Among these, interstitial fibrosis is regarded as a pivotal factor for arrhythmic development [2]. Our previous study on a transgenic (TG) mouse strain revealed development of cardiomyopathy and premature death [3]. Myocardial fibrosis is the most prominent histopathology in this model [2,3]. We recently demonstrated enhanced oxidative stress in the TG mouse heart leading to fibrosis, cardiomyocyte hypertrophy/apoptosis and inflammation [4], and treatment with the antioxidant N-acetylcysteine (NAC) reduced fibrosis and myocyte death and improved cardiac function [4]. These findings support the view that increased oxidative stress in a failing heart is a pivotal molecular mechanism of histopathology, particularly fibrosis [5].

We very recently observed frequent onset of ventricular tachy-arrhythmias in the TG mice (our unpublished data). This TG strain represents an ideal model for pre-clinical therapeutic testing. This project will extend our previous study [4] to test if antioxidant therapy is able to reduce the severity of arrhythmias. TG mice will receive treatment with NAC (0.5 mg/kg/day) for one month. During the treatment period, animals will be studied by echocardiography to determine cardiac function and by telemetry technique to monitor development of ventricular arrhythmias. Another group of TG mice will be similarly studied for comparison. At the end of the treatment period, changes in cardiac fibrosis and degree of cardiac dilatation will be related to ventricular arrhythmias.

Project related methods/skills/technologies:

- Echocardiography for cardiac functional assessment
- Telemetry recording of electrocardiogram for determination of arrhythmias
- Biochemical assays and quantitative histology
- Drug administration, animal monitoring

References:

Project title: Role of macrophage-migration inhibitory factor, MIF, in activating systemic inflammation post myocardial infarction

Laboratory: Experimental Cardiology

Primary Supervisor (s)       Xiaoming Gao,   Xiao-Jun Du

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Research Focus: Myocardial infarction (MI) is associated with significant inflammatory response leading to additional myocardial injury and cardiac remodeling. Our recent studies have identified MIF as a pivotal cytokine in promoting inflammatory response. This study will characterize the role of MIF in systemic and cardiac inflammation following MI.

Keywords: macrophage migration inhibitory factor, myocardial infarction, inflammation,

Project description:

Myocardial infarction (MI) is the leading cause of cardiac death worldwide. Following prolonged ischemia, necrotic cardiomyocytes release a range of inflammatory molecules. These molecules trigger systemic inflammatory response which promotes recruitment of leukocytes to the injured myocardium thereby facilitate healing. However, excessive inflammatory response leads to further tissue damage and adverse outcomes. Macrophage migration inhibitory factor (MIF) is a well known inflammatory cytokine, which plays a pivotal role in various inflammatory disorders, including MI. We hypothesize that MIF may play a critical role in activating systemic inflammatory responses following MI. Using a mouse strain with genetic deletion of MIF gene compared to wild type control mice, we will first study the influence of MIF on activation of circulating inflammatory cells by measuring hematology profile, expression of pro-inflammatory mediators in peripheral blood mononuclear cells (PBMCs) and analyzing population of circulating pro-inflammatory monocytes with Flow cytometry (FACS). As spleen is the largest lymphoid organ in the body and contributes to 50% of infiltrated monocytes in the infarct myocardium. Second, we will explore the influence of MIF on mobilization of splenic monocytes by measuring spleen weigh, and changes in splenic tissue architecture and in population of pro-inflammatory monocyte in the spleen with FACS. Third, we will also study the changes of above mentioned systemic inflammatory parameters after anti-MIF intervention in wild type mice to explore the therapeutic potential. This project will help for better understanding the role MIF in ischemic-mediated inflammation which may lead to a better management of MI.

Project related methods/skills/technologies:

- Production of animal model of myocardial infarction
- Flow cytometry
- Biochemical assays and quantitative histology
- Drug administration, animal monitoring

References:

1. Dayawansa N, Gao XM, White D, Dart AM, Du XJ. Role of MIF in acute myocardial ischemia and infarction: insight from recent clinical and experimental findings. (invited review) Clinical Science 2014;127(3):149-161

### Project title: Identification of microRNA networks in cardiac diseases

**Laboratory:** Cardiac Hypertrophy

**Primary Supervisor (s)** Dr Jenny Ooi, A/Prof Julie McMullen

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**Research Focus:** Bioinformatics approaches for cardiac disease

**Keywords** Cardiac hypertrophy, bioinformatics, microRNAs

**Project description:**

Heart failure is a major clinical problem affecting 1-3% Australians, therefore strategies to protect the heart against insults that lead to heart failure such as pathological cardiac hypertrophy (growth of the heart) is becoming more critical. MicroRNAs (miRNAs) are a family of small RNAs that play important roles in the regulation of target genes by interacting/binding with specific sites in 3' untranslated regions of messenger transcripts to repress their translation or regulate degradation.

Our laboratory has successfully regulated one of these miRNAs with an RNA-based therapy in a mouse model with cardiac dysfunction, and demonstrated improved outcome and reduced pathology with this treatment strategy (1, 2). We have collected tissues from these mice and with the development and improvement of deep sequencing technologies we now have the opportunity to identify and comprehensively assess the role of other small non-coding RNAs in addition to miRNAs simultaneously (3).

This project will use bioinformatics approaches to investigate changes in other small RNAs and build miRNA-associated networks that will improve our understanding of the pathways that regulate complex diseases like heart failure. After identification of key miRNAs, the student will use molecular and cell culture experimental techniques to perform validations.

**Project related methods/skills/technologies:**

- Bioinformatics
- Molecular techniques

**References:**

**Project title:** Failing heart, failing kidney: novel treatment approaches

**Laboratory:** Heart Failure Research Group (head: David Kaye)

**Primary Supervisor(s)** Dr Niwanthi Rajapakse (niwanthi.rajapakse@bakeridi.edu.au) and Professor David Kaye (david.kaye@bakeridi.edu.au)

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**Research Focus:**
Heart Failure, kidney failure

**Keywords** heart failure, kidney failure, Kidney fibrosis

**Project description:**

Development of kidney failure is a complication suffered by many patients with heart failure (HF). Importantly, when these two conditions occur simultaneously, patients can develop fluid retention leading to poor survival. Yet, there are no specific treatments for HF related renal dysfunction. The mechanism that leads to the onset of renal failure in HF is complex, and involves a combination of renal fibrosis and poor blood supply to the kidney. Our study will investigate the cause of renal fibrosis in a clinically relevant transgenic mouse model of HF. We will also test whether a novel drug intervention can improve renal function in HF patients with severe kidney impairment. This clinical trial seeks to develop a new treatment to improve kidney function in HF patients, that could rapidly progress to clinical practice. Students can choose from projects involving cell culture experiments/ animal experiments and/or experiments relating to clinical studies in patients.

**Project related methods/skills/technologies:**

- Animal surgery
- Biochemical techniques (westerns, PCR)
- Assays in plasma samples collected from patients

**References:**

Project title: Circulating microRNAs as novel biomarkers for acute myocardial ischemia

Laboratory: Vascular Pharmacology

Primary Supervisor(s)
Prof. Anthony Dart, Dr. Lu Fang, Prof. Jaye Chin-Dusting

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Research Focus:
We aim to evaluate circulating microRNAs as a rapid and sensitive marker for acute myocardial ischemia

Keywords: myocardial ischemia, microRNAs, biomarkers

Project description:
Coronary artery disease and acute myocardial infarction (AMI) are the leading cause of death worldwide. Over the last few decades, measurement of circulating biomarkers has become important in the diagnosis of acute chest pain. Cardiac troponin, which rises at about 3.5 h after the onset of chest pain in AMI patients, is a diagnostic marker for AMI. However, there are currently no rapid and easily assessed markers for acute myocardial ischemia.

MiRNAs are short, noncoding RNAs of 18-25 nucleotides that posttranscriptionally control gene expression.1 Circulating miRNAs, released by cells into circulation, are potential novel biomarker for cardiovascular diseases such as AMI, heart failure, and hypertension.2, 3 Recent studies suggest that circulating miRNAs rise earlier than cardiac troponin. In animal models with AMI, circulating miRNAs elevated 15-30 min after ischemia (insufficient to lead to cardiac cell death),4 suggesting that myocardial ischemia less than 15 min may be a sufficient stimulus for cardiac release of miRNAs.

This project is aimed to study whether measurement of plasma miRNA profile, as a non-invasive method, help to identify acute chest pain patients with ischemic heart disease from non-ischemic heart disease and if so whether they will also identify the extent of myocardial ischemia.

We will take blood from chest pain patients referred to stress nuclear medicine imaging. Myocardial ischemia will be diagnosed by stress nuclear perfusion test. Plasma miRNAs will be compared between patients with chest pain due to ischemia or non-ischemia. The relation between miRNA levels and the degree of ischemia will be assessed.

Project related methods/skills/technologies:
- Recruiting patients with chest pain from Alfred Hospital and collect blood samples
- Performing assays, such as isolating RNA from plasma samples, performing real-time PCR, Elisa etc
- Analyzing data and performing statistical tests

References:
**Project title: The role of phospholipids in macrophage polarization**

**Laboratory:** Vascular Pharmacology

**Primary Supervisor(s)**

Prof. Jaye Chin-Dusting, Dr. Lu Fang, Dr Shirley Moore

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**Research Focus:**

Macrophage polarization

**Keywords:** macrophage polarization, phospholipids, inflammation

**Project description:**

Macrophages play an important role in the initiation, development and instability of atherosclerotic plaques. In the earliest stage of atherosclerosis, macrophages engulf oxidized low-density lipoprotein (LDL) and become foam cells, which is the hallmark of atherosclerosis. Foam cells, in turn, contribute to continuous recruitment of circulating monocytes, advancement of atherosclerosis and plaque vulnerability by secreting a variety of pro-inflammatory cytokines, chemokines, and proteases. Macrophages can be polarized with different stimuli into two distinct subsets: classically-activated (M1) macrophages and alternatively-activated (M2) macrophages. M1 macrophages have pro-inflammatory phenotype and have the capacity to sustain inflammation by secreting inflammatory cytokines. On the other hand, M2 macrophages are anti-inflammatory and promote tissue repair and healing.  

Unlike LDL, high-density lipoprotein (HDL) has atheroprotective function and is inversely associated with the risk of cardiovascular disease. HDL is known to exert atheroprotective action through promoting reverse cholesterol transportation and inhibiting inflammatory response. While the majority studies on HDL focus on Apo-A1, the protein component of HDL, there is accumulating evidence pointing to the importance of lipid component of HDL. Phospholipids (PL) is a major lipid component of HDL. Epidemiological studies have found that the severity of coronary artery disease is correlated with low levels of HDL-PL. Several studies have also shown that PL of HDL plays a role in reverse cholesterol efflux. Our previous work has shown that PL promotes M2 but inhibits M1 macrophage polarization. Next, we aim to further explore the signalling pathways by which PL mediates its actions.

**Project related methods/skills/technologies:**

- Blood monocyte isolation and cell culture
- Technique of cellular and molecular biology, such as flow cytometry, real-time PCR, Western blot, cell imaging, etc
- data analysis and statistical tests

**References:**

# Project title: New Cellular Cholesterol Transporter

**Laboratory:** Lipoproteins and Atherosclerosis

**Primary Supervisor (s):** Prof. Dmitri Sviridov, Dr. Ying Fu

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**Research Focus:** Treatment for atherosclerosis and diabetes

**Keywords:** Atherosclerosis, lipids, diabetes, heart disease, vascular biology

## Project description:

Atherosclerosis is the cause of majority of cardiovascular diseases, a major cause of death in Western societies. Atherosclerosis is essentially accumulation of excessive cholesterol in the walls of arteries with the formation of atherosclerotic plaque blocking the blood flow and causing thrombosis. Accumulation of cholesterol may be caused by excessive delivery of cholesterol from blood or by damaged pathways responsible for eliminating excess of intracellular cholesterol. Disturbances in intracellular cholesterol metabolism are the primary cause of impairment of cholesterol release and are on the full front of rapidly emerging anti-atherosclerotic therapies. Cholesterol homeostasis also plays a key role in diabetes: accumulation of cholesterol in β-cells severely disrupts insulin secretion.

A key element of intracellular cholesterol metabolism is a group of proteins moving cholesterol around the cell called ABC transporters. ABC transporters regulate release of cholesterol from cells to plasma and maintain correct intracellular cholesterol content. Surprisingly, very little is known about how these transporters work.

We have recently discovered that one of the transporters, ABCA12, which was known to play an important role in skin, also plays an important role in macrophages, cell central for development of atherosclerosis and inflammation and in β-cells. It appears that ABCA12 is responsible for “regulating the regulator” – modulating a major pathway responsible for coordinate action of other ABC transporters. The same pathway is also involved in regulation of inflammation. The study is a combination of sophisticated *in vitro* study aimed at discovering the molecular and cellular mechanisms of how ABCA12 regulates this pathway, and *in vivo* study aimed at testing the effects of ABCA12 deficiency on development of atherosclerosis and diabetes in mouse model of these diseases. The project is conducted in collaboration with Monash University.

## Project related methods/skills/technologies:

- Cell biology
- Animal models

## References:

Project title: Cholesterol metabolism and complications of HIV disease

Laboratory: Lipoproteins and Atherosclerosis

Primary Supervisor(s): Prof. Dmitri Sviridov, Dr. Nigora Mukhamedova

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Research Focus: Pathogenesis of complications of HIV disease

Keywords: HIV, atherosclerosis, lipids, diabetes, heart disease, vascular biology

Project description:

Current treatment for HIV infection has dramatically reduced mortality, however, co-morbidities that are not directly related to immunodeficiency are now increasingly recognized as a consequence of HIV infection. One such co-morbidity is an increased risk of cardiovascular disease. The current view is that HIV infection and/or its treatment are associated with elevated risk of development of atherosclerosis and consequently with increased prevalence of acute and chronic cardiovascular events. HIV also causes disturbances of lipoprotein metabolism, metabolic syndrome, lipodystrophy, dementia.

We currently investigate how HIV is causing co-morbidities in the organs not infected with the virus. Our hypothesis is that HIV-infected cells release viral proteins and MiRs in the bloodstream that affect uninfected cells causing pathology in these cells without infection. Our study is focused on establishing what factors are released by HIV-infected cells and which pathways in uninfected cells are affected. Our hypothesis supported by large volume of data is that the pathways affected are pathways related to cholesterol metabolism.

The project is a combination of in vitro work in cell culture, animal studies and some clinical work. It is on the crossroads of virology and cardiology and gives an opportunity to learn a wide range of techniques, from cell biology to biochemistry as well as clinical studies. The project is conducted in collaboration with a number of Australian and overseas laboratories and gives the participants an exposure to research in various disciplines.

Project related methods/skills/technologies:

- Cell biology
- Animal models

References:

**Project title:** Apolipoprotein A-I mimetic peptides and Treatment for Heart Disease

**Laboratory:** Lipoproteins and Atherosclerosis

**Primary Supervisor (s):** Prof. Dmitri Sviridov, Dr. Michael Ditiatkovski

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**Research Focus:** Treatment for atherosclerosis

**Keywords:** Atherosclerosis, lipids, heart disease, vascular biology, animal models

**Project description:**

Drugs affecting lipid metabolism have revolutionized treatment of atherosclerosis reducing the risk of cardiovascular diseases by 30-40%. There is, however, an urgent need for further reduction of the unacceptably high remaining risk of CVD. A most promising direction is complementing decreasing levels of the pro-atherogenic lipoproteins (“bad cholesterol”) with increasing levels of the anti-atherogenic lipoprotein, high density lipoprotein (HDL, “good cholesterol”), i.e. “HDL Therapy”. The efficiency of HDL therapy critically depends on the method used to for elevate HDL levels. A promising type of HDL therapy is the use of peptides mimicking the structure of the main protein of HDL, apolipoprotein A-I (apoA-I). One advantage of these peptides is that their structure can be easily changed to fine-tune their properties. In the lead up to this project we developed a series of peptides that mimic various anti-atherogenic properties of HDL. The project has two aims. One is to use various peptides that have one, but not other anti-atherogenic properties of HDL to determine which properties are most important for the protection against atherosclerosis. The second aim is to develop a peptide, or a combination of peptides which manifest most significant anti-atherogenic properties of HDL and can be further developed into a drug. The project is substantially animal based, involving the infusion of peptides into a mouse model of atherosclerosis to examine their effect on atherosclerotic plaques. In addition this study will include a sizable in vitro component; where the effect of peptides on plasma lipoproteins is investigated. The study is conducted in close collaboration with National Institutes of Health, USA.

**Project related methods/skills/technologies:**

- Cell biology
- Animal models

**References:**


Project title: Plasmalogen modulation as a treatment for atherosclerosis

Laboratory: Metabolomics

Primary Supervisor(s): A/Prof Peter Meikle; Dr Judy DeHaan

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Research Focus: Metabolomics is the systematic study of the unique metabolite (small-molecule) fingerprints of biological systems. The Metabolomics Laboratory uses “state of the art” tandem mass spectrometry to obtain metabolic/lipid profiles from cell and animal models in addition to clinically relevant human samples to develop new approaches to early diagnosis, risk assessment and therapeutic monitoring of diabetes and cardiovascular disease [1]. This approach is also combined with cell biology and animal studies to investigate lipid metabolism and pathogenesis in atherosclerosis and other disease states. The Metabolomics Laboratory has a number of project areas that are suitable for Honours, Masters and PhD programs.

Keywords: Heart disease; atherosclerosis, therapy, lipids, plasmalogens,

Project description: Atherosclerosis (AS) is the single most common cause of cardiovascular disease and is the major contributor to the development of angina, heart attacks, congestive heart failure, peripheral vascular disease and stroke. We have performed detailed lipidomic analysis of plasma and lipoproteins from healthy individuals as well as stable and unstable coronary artery disease (CAD) patients. We identified associations of phosphatidylcholine plasmalogens with stable CAD (relative to healthy control individuals) and of phosphatidylethanolamine plasmalogens with unstable CAD (relative to stable CAD) [2]. We have supplemented the diet of ApoE mice with batyl alcohol (a plasmalogen precursor); this resulted in a four-fold increase in plasma levels of plasmalogens and a significant attenuation of plaque formation (average 70%) across all regions of the aorta.

Figure. Plasmalogen supplementation prevented plasmalogen progression in ApoE deficient mice. Representative en face aortic images, showing arch, thoracic, and abdominal sections from C57/BL6 and ApoE KO mice with and without 2% BA treatment for 12 weeks. Areas stained in red are atherosclerotic plaques.

We hypothesize that: Upregulation of plasmalogens prevents plaque formation by multiple mechanisms, including: 1) alteration of lipoprotein structure and function; 2) reduction in oxidative stress in tissue beds; and 3) suppression of the inflammatory response leading to a reduction of the monocyte-endothelium interaction.

In this project we will combine our lipidomics and lipoprotein expertise with our established cell and mouse models of oxidative stress, atherosclerosis and unstable plaque to define the mechanism(s) of plasmalogen attenuation of plaque and to define the effect on plaque regression and stability.
The specific aims are to:

1) **Optimise the plasmalogen modulation protocol**
2) **Characterise the mechanisms by which plasmalogens attenuate plaque progression**
3) **Assess the capacity of plasmalogens to regress plaque.**
4) **Determine the ability of plasmalogens to prevent plaque instability**

This project will provide a mechanistic understanding of the effect and extent of plasmalogen modulation on atherosclerosis and identify suitable endpoints for clinical trials. 

*Importantly, up-regulation of plasmalogen levels in animals and humans can be achieved using oral administration of the natural compounds (alkylglycerols) and so progression into clinical trials will be rapid and safe.*

*This project would be suitable for a PhD student or a sub project could be identified for an Honours student.*

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**Project related methods/skills/technologies:**

- Lipid extraction and analysis by mass spectrometry
- Cell culture experiments
- Animal experiments

**References:**

**Project title:** High Density Lipoprotein and Oxidized Lipids in the Prediction of Acute Coronary Syndromes

**Laboratory:** Metabolomics

**Primary Supervisor (s):** A/Prof Peter Meikle; Prof Bronwyn Kingwell

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**Research Focus:** Metabolomics is the systematic study of the unique metabolite (small-molecule) fingerprints of biological systems. The Metabolomics Laboratory uses “state of the art” tandem mass spectrometry to obtain metabolic/lipid profiles from cell and animal models in addition to clinically relevant human samples to develop new approaches to early diagnosis, risk assessment and therapeutic monitoring of diabetes and cardiovascular disease [1]. This approach is also combined with cell biology and animal studies to investigate lipid metabolism and pathogenesis in atherosclerosis and other disease states. The Metabolomics Laboratory has a number of project areas that are suitable for Honours, Masters and PhD programs.

**Keywords:** Heart disease; atherosclerosis, lipoprotein, lipids, mass spectrometry, biomarker

**Project description:** Worldwide more than 19 million people per annum experience a sudden cardiac event including sudden death, non-fatal myocardial infarction or unstable angina. However, it is still not possible to accurately identify those at risk of coronary plaque rupture or erosion, the major underlying causes. This proposal builds on our patented whole plasma lipidomic profile which identified patients experiencing an unstable or acute coronary syndrome from stable coronary artery disease (CAD) patients significantly better than conventional risk factors [2].

Our preliminary data shows that atherosclerotic plaque contains a high proportion of oxidized lipids and that these lipids can be taken up by high density lipoproteins (HDL).

**We hypothesize that:**

1) Oxidized lipids, produced in plaque, will be exported from plaque into circulation on HDL particles.

2) Lipidomic profiles of HDL incorporating these oxidised lipids will add significantly to traditional risk factors to better predict future plaque rupture and acute (unstable) coronary syndromes.

In this project we will tandem mass spectrometry to characterize the lipid composition of atherosclerotic plaque and identify new lipid markers. Clinically defined patient samples will be used to isolate lipoproteins from whole plasma using ultracentrifugation. We will then analyse the composition and distribution of the newly identified lipids across the lipoprotein pools and relate these lipid profiles to stages of disease.

**The specific aims are to:**

1) To identify and characterise novel oxidised lipids within carotid endarterectomy specimens.

2) To incorporate these lipids into our high throughput lipidomic assays.

3) To evaluate the lipidomic profile of HDL in patients presenting with stable or unstable CAD.

A secondary aim is to understand the relationship between HDL lipid composition and various aspects of HDL function in the context of plaque rupture/erosion. These include cholesterol efflux, anti-oxidative and anti-inflammatory properties. Studies in this area will provide insight into the compelling epidemiological evidence that HDL is atheroprotective, and establish a basis for identification of biomarkers of both risk and therapeutic response.

**This project would be suitable for a PhD student or a sub project could be identified for an Honors student.**
### Project related methods/skills/technologies:

- LDL/HDL preparation from plasma
- Lipid analysis and characterization by tandem mass spectrometry
- HDL functionality assays

### References:


Project title: Plasma Lipid Profiling in Type 2 Diabetes and Coronary Artery Disease

Laboratory: Metabolomics

Primary Supervisor(s): A/Prof Peter Meikle; Dr Chris Barlow.

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Research Focus: Metabolomics is the systematic study of the unique metabolite (small-molecule) fingerprints of biological systems. The Metabolomics Laboratory uses “state of the art” tandem mass spectrometry to obtain metabolic/lipid profiles from cell and animal models in addition to clinically relevant human samples to develop new approaches to early diagnosis, risk assessment and therapeutic monitoring of diabetes and cardiovascular disease [1]. This approach is also combined with cell biology and animal studies to investigate lipid metabolism and pathogenesis in atherosclerosis and other disease states. The Metabolomics Laboratory has a number of project areas that are suitable for Honours, Masters and PhD programs.

Keywords: Heart disease; atherosclerosis, lipoprotein, lipids, mass spectrometry, biomarker

Project description: Type 2 diabetes and coronary artery disease are major causes of morbidity and mortality in Australia. A number of lipids and lipoproteins have been identified as useful indicators and predictors of both type 2 diabetes and atherosclerosis (i.e. cholesterol, HDL, triglycerides). However, these provide only a restricted picture and a limited interpretation of the disease risk/status of an individual. It is now becoming clear that many other lipid types are altered during disease onset and progression and it is likely that some/many of these are involved in disease pathogenesis. We have an ongoing program of method development and biomarker discovery to identify and validate new lipid biomarkers of disease [2, 3].

In this project we will use our novel lipidomic approach to generate lipid profiles from patient cohorts at different stages of disease to identify those lipids and lipid profiles that are specifically associated with disease onset and progression. We hypothesize that: the major differences in the plasma lipid profiles between healthy and type 2 diabetes or coronary artery disease precede the clinical presentation and so will be useful to predict disease outcomes.

The specific aims are to:

1) Perform plasma lipid profiling on patient cohorts that have been clinically phenotyped (type 2 diabetes or coronary artery disease).

2) Determine the plasma lipid profiles that are correlated with the burden of disease and use this to develop predictive models to identify individuals at increased risk of a disease onset and progression.

The primary outcome of this project will be the development of a plasma lipid profiling test to enable the early detection of patients at increased risk of type 2 diabetes and coronary artery disease. In addition we will develop methods to monitor treatment. Identification of individuals prior to the development of disease will enable early intervention and will have a profound effect on the health of the Australian population.

This project would be suitable for a PhD student or a sub project could be identified for an Honors student.
## Project related methods/skills/technologies:

- Assay development;
- Lipid analysis by electrospray ionisation mass spectrometry;
- Statistical analysis.

## References:


Project title: Stable isotope labeling for quantitative lipidomics of fatty acid treated liver cells.

Laboratory: Metabolomics

Primary Supervisor (s) A/Prof Peter Meikle; Dr Christopher Barlow

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Research Focus: Metabolomics is the systematic study of the unique metabolite (small-molecule) fingerprints of biological systems. The Metabolomics Laboratory uses “state of the art” tandem mass spectrometry to obtain metabolic/lipid profiles from cell and animal models in addition to clinically relevant human samples to develop new approaches to early diagnosis, risk assessment and therapeutic monitoring of diabetes and cardiovascular disease. This approach is also combined with cell biology and animal studies to investigate lipid metabolism and pathogenesis in atherosclerosis and other disease states. The Metabolomics Laboratory has a number of project areas that are suitable for Honours, Masters and PhD programs.

Keywords Mass Spectrometry, Lipidomics, Stable isotope labeling (SILAC)

Project description:
Large scale high-throughput lipidomics has the potential to provide new insights into the molecular pathology of disease as well as identifying biomarkers for early diagnosis, risk assessment and therapeutic monitoring of diabetes and cardiovascular disease. Electrospray ionization mass spectrometry (ESI-MS) is the enabling technology which has underpinned the rapid growth in lipidomics. Unfortunately there are still significant analytical challenges associated with reproducibility and accurate quantitation in ESI-MS based analysis. The gold standard for MS quantitation is the use of stable isotope labeled standards, however limited standards are available commercially and are often extremely expensive. In the related field of proteomics, SILAC1 (stable isotope labeling by amino acids in cell culture) has been used to overcome these difficulties. Here we plan to apply a similar strategy for lipidomics. By growing cells in media with appropriate isotopic labeled substrates it is possible to use these cells to produce isotopically labeled lipids in relatively high purity which may then be isolated and used as internal standards.

We hypothesize that:
1. Appropriate conditions can be found in which cells may be grown to produce isotopically labeled lipids in purity exceeding 95% isotopic enrichment.
2. Inclusion of isotopically labeled standards will significantly reduce the analytical variation in lipidomic analysis over time and across instrument platforms.

To evaluate the new methodology, we will use these standards to assess the effect of elevated fatty acids on the lipidome in liver cells.

This project would be suitable for an Honours student.

Project related methods/skills/technologies:
- Cell Culture
- Liquid chromatography
- Mass spectrometry and lipidomics

References:
**Project title: High-density lipoprotein (HDL) and cardiac metabolism**

**Laboratory:** Metabolic & Vascular Physiology

**Primary Supervisor (s):** Andrew Siebel & Bronwyn Kingwell

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**Research Focus:**
Study of the effects of HDL on cardiac glucose metabolism in the setting of type 2 diabetes

**Keywords:**
Heart disease, glucose metabolism, cardiac function, myocardial ischaemia, type 2 diabetes

**Project description:**
High-density lipoprotein (HDL) is best known for its anti-atherosclerotic actions, which have prompted development of HDL-raising strategies to combat cardiovascular disease. We have characterized previously unrecognized mechanisms by which HDL modulates glucose metabolism in both skeletal muscle and the pancreas.

This project extends our discoveries to examine the effects of HDL on myocardial glucose metabolism following ischaemia, using cardiac muscle cell culture and in vivo mouse models. We will also determine whether these effects are mediated via the Akt-signalling pathway in the presence and absence of insulin resistance and type 2 diabetes. Given the escalating prevalence of obesity and type 2 diabetes, and the associated cardiovascular complications, understanding mechanisms which may lead to new therapeutic approaches targeting myocardial metabolism and function has potential for significant impact.

Opportunities exist for Honours, Masters and PhD students.

**Project related methods/skills/technologies:**
- Cell culture
- Western blotting, qPCR, radiolabelled assays
- In vivo animal handling, surgery, functional analyses and tissue collection

**References:**
### Project title: Development of brown adipose tissue for treatment of obesity

**Laboratory:** Metabolic & Vascular Physiology

**Primary Supervisor(s):** Andrew Carey & Bronwyn Kingwell

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**Research Focus:**
Study of brown adipose (fat) in humans as a potential treatment for obesity

**Keywords:**
Obesity, diabetes, fat, brown fat, brown adipose tissue, energy expenditure

**Project description:**
Fundamentally, obesity results from an imbalance between energy intake and expenditure. Current preventative and therapeutic approaches have been either unsustainable or result in significant negative side effects. Brown adipose tissue (BAT) is unique with respect to its sole function of burning potentially great quantities of energy, therefore increasing BAT content and activity is currently considered one of the most promising strategies to increase energy expenditure to combat obesity. BAT function in small animals is well described, however in humans knowledge is limited due to only recently being conclusively identified in adults and the identification of novel techniques to measure its activity.

Our ongoing studies therefore provide opportunities to explore the possibility of combating obesity related disease while gaining broad research experience and skills ranging from clinical research to numerous wet lab techniques.

**Project related methods/skills/technologies:**
Numerous aspects of human clinical research, including
- Volunteer recruitment
- Tissue collection
- Body composition analysis
- Measurement of whole body energy expenditure
- Examination of nuclear medicine scans for BAT activity
- Basic Science/wet laboratory analytical techniques
- Opportunity to discuss your work regularly to both lay people and those involved in research

**References:**
**Project title: Eplerenone in the management of abdominal aortic aneurysm (AAA)**

**Laboratory:** Metabolic & Vascular Physiology

**Primary Supervisor(s):** Anna Ahimastos & Bronwyn Kingwell

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**Research Focus:**
Investigation of the efficacy of eplerenone in the reduction of AAA growth

**Keywords:**
Clinical trial, abdominal aortic aneurysm, mineralocorticoid receptor antagonism

**Project description:**
Abdominal aortic aneurysm (AAA) is responsible for approx. 1,500 deaths and 10,000 hospital admissions per year in Australia. Surgery is the only therapy for AAA but is costly and associated with high morbidity and mortality. No medical therapy has been approved for AAA, highlighting the need for a better understanding of its pathophysiology to implement novel management strategies.

A growing body of clinical and animal data implicate activation of the mineralocorticoid receptor (MR) in the pathogenesis of AAAs. MR antagonism represents a potential strategy to reduce AAA growth. Eplerenone, a MR antagonist, is currently indicated for heart failure, and has been shown to have anti-fibrotic effects in both the heart and aorta. Importantly, it has been demonstrated that administration of eplerenone to an AAA mouse model completely blunted aneurysm formation. Whether these exciting preliminary findings translate into a new therapy for AAA patients, needs to be explored.

The aims of the proposed clinical trial are to investigate the efficacy of eplerenone to:
1. Reduce AAA growth assessed by magnetic resonance imaging
2. Reduce circulating concentrations of pro-aneurysmal biomarkers.

This will be the first human clinical trial to provide evidence of whether eplerenone is effective in limiting AAA growth, and potentially averting or delaying complications, including expensive surgery. This trial will also provide important mechanistic insights regarding vascular mechanisms of action of eplerenone, and will lead to identification of potential biomarkers of AAA progression which will enable more effective clinical monitoring. This work has significant potential to improve clinical management of patients with small AAAs.

**Project related methods/skills/technologies:**
- Clinical skills, including volunteer recruitment and patient interaction
- Basic Science/wet laboratory analytical techniques

**References:**
### Project title: Peripheral artery disease, glucose metabolism and skeletal muscle blood flow

**Laboratory:** Metabolic & Vascular Physiology

**Primary Supervisor(s):** Julian Sacre & Bronwyn Kingwell

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**Research Focus:**
Study to determine whether treatment of insulin resistance by metformin may be a novel and effective strategy to improve blood flow reserve and exercise tolerance in people with peripheral artery disease.

**Keywords:**
clinical trial, peripheral artery disease, skeletal muscle blood flow, glucose metabolism, insulin resistance, metformin

**Project description:**
Peripheral artery disease is characterized by stenosis/occlusion of the arteries in the legs, which limits blood flow responses to exercise. This blunting of blood flow reserve can manifest clinically as leg ischaemic pain on walking (“claudication”) – a symptom that is often responsible for marked loss of exercise capacity/mobility in patients. The Baker IDI Metabolic & Vascular Physiology Laboratory has an ongoing research program directed to identifying novel pharmacologic treatments capable of alleviating this ischaemic leg pain and improving exercise tolerance.

We have recently commenced a clinical trial in peripheral artery disease to determine whether treatment of glucose metabolism / insulin resistance by the drug ‘metformin’ (commonly used in type 2 diabetes) may be a novel and effective strategy to improve blood flow reserve and exercise tolerance. This and/or related projects provide opportunities for students with an interest in exercise and mechanistic physiology.

**Project related methods/skills/technologies:**
- Clinical research techniques (i.e. leg blood flow, endothelial function assessment, exercise testing, etc)
- Broader research experience would also be gained via the recruitment, screening, and testing of study participants
- Data analysis
## Project title: Role of the Central Nervous System in Obesity related hypertension

### Laboratory:
Neuropharmacology laboratory

### Primary Supervisor (s):
Prof Geoff Head, Dr Joon Lim (Kyungjoon Lim) & Dr Pamela Davern

### Contact:
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### Research Focus:
The influence of the central nervous system on obesity related hypertension and the relationship between trans-generational effect of obesity and blood pressure. Research in Neuropharmacology centres on cardiovascular neuroscience and fills a niche between the clinic and basic research.

### Keywords
- Obesity, Hypertension, Developmental Programming, Brain, Physiology, Pharmacology, Leptin

### Project description:
Cardiovascular disease and its ultimate consequences are a significant burden on health systems in Australia and around the developed world. It is increasingly apparent that the high rate of hypertension in many societies can be attributed to an equally alarming rate of obesity. Despite this link, there is a less than complete understanding of the manner by which obesity alters central nervous system function to result in hypertension.

It is known that the sympathetic nervous system (SNS), responsible in part for the control of blood pressure and is overactive in obese humans. It is also known that several peripheral signalling chemicals act at specific brain sites to modulate both appetite and also the activity of the SNS. For example, leptin, produced by fat and responsible for reducing appetite by acting at specific sites in the brain may act also to either stimulate or inhibit SNS activity depending on which neurons it acts at in the hypothalamus. Nonetheless, despite a comprehensive understanding of changes in appetite systems in the brain in obesity, and a known link between some of these appetite centres and other nuclei that control SNS outflow there is little understanding of how the sympathetic nervous system is activated during the development of obesity.

### Project aims:
We seek to determine the manner by which various brain centres respond in obesity by studying patterns of neural activation and measuring SNS activity and blood pressure during the development of obesity using rabbits.

### Project related methods/skills/technologies:
- Animal handling and surgery
- Immuno-histochemical analysis of brain regions
- Direct measurement of blood pressure and renal sympathetic nerve activity in conscious rabbit

### References:
1. Kyungjoon Lim, Sandra L. Burke and Geoffrey A. Head (2013). “Obesity related hypertension and the role of insulin and leptin in high fat fed rabbits” *Hypertension* 61; 628-634, selected for evaluation by Faculty of 1000 (F1000), Top 2 % in all Biological & Medical Journals in the world).
2. Kyungjoon Lim, Sandra L. Burke, James A. Armitage, Geoffrey A. Head (2012). “Comparison of blood pressure and sympathetic activity of rabbits in their home cage and the laboratory environment” *Experimental Physiology* 97 (12):1263, selected for evaluation by Faculty of 1000 (F1000), Top 2 % in all Biological & Medical Journals in the world).
**Project title:** Role of the brain in a mouse model of neurogenic hypertension  

**Laboratory:** Neuropharmacology laboratory  

**Primary Supervisor(s):** Prof Geoff Head, Dr Kristy Jackson & Dr Pamela Davern  

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**Research Focus:**  
The influence of the central nervous system on long-term blood pressure levels and the relationship between blood pressure and stress pathways in the brain is a major focus of the Neuropharmacology Lab’s studies. Research in Neuropharmacology centres on cardiovascular neuroscience and fills a niche between the clinic and basic research.  

**Keywords:** Hypertension, brain, immunohistochemistry, pharmacology  

**Project description:**  
The Schlager BPH/2J mice is a model of hypertension which is caused by overactivity of the sympathetic nervous system (SNS) driven by greater activity of neurons in the medial amygdala, a brain region most notable for its role in stress. The brain regions downstream of the medial amygdala which result in SNS overactivity likely include projections to the hypothalamus but to date these pathways have not been assessed.  

**Part 1** of this student project will determine these downstream brain pathways involved in causing the hypertension in BPH/2J mice. This will be achieved by injecting a tracing compound into the medial amygdala and tracking where in the brain these neurons project using immunohistochemistry. In particular we will identify brain regions involved in hypertension by colocalising with another immunohistochemical marker of neuronal activity (Fos).  

**Part 2** of this student project will be to pharmacologically inhibit one of the important brain regions (identified in part one of this study) and measure the effect on blood pressure in conscious BPH/2J mice via radiotelemetry probes.  

**Project related methods/skills/technologies:**  
- Animal handling and surgery  
- Immuno-histochemical analysis of brain regions  
- Direct measurement of blood pressure in conscious freely moving mice  

**References:**  
## Project title: Hypertension induced by exposure of chronic stress and a high fat diet

**Laboratory:** Neuropharmacology laboratory

**Primary Supervisor(s):** Prof Geoff Head, & Dr Pamela Davern

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**Research Focus:**

The influence of the central nervous system on long-term blood pressure levels and the relationship between blood pressure and stress pathways in the brain is a major focus of the Neuropharmacology Lab’s studies. Research in Neuropharmacology centers on cardiovascular neuroscience and fills a niche between the clinic and basic research. Work is carried out to understand the mechanisms that trigger cardiovascular diseases through environmental factors. Stress is a main area of investigation, and research is also being conducted on the effects on the central nervous system and control of the cardiovascular system of obesity and other metabolic disorders.

**Keywords:** Hypertension, Chronic stress, Obesity, Central nervous system

**Project description:**

The effects of acute stress have been well documented in the literature but the mechanisms by which chronic stress or repeated daily exposure to acute stress contributes to sustained elevations in blood pressure is not well understood. Obesity rates are ever increasing and the highest rates are from sedentary individuals who continue to be exposed to stressful events over the long term. Metabolic syndrome is comprised of a combination of obesity, hypertension, and cholesterol levels outside the acceptable range; and therefore is a major risk factor for cardiovascular disease. Our laboratory has shown that a high fat diet increases blood pressure via activation of specific neurons in the brain to increase nerve activity to the kidney. Interestingly we discovered that these neurons are also those responsible for the hypertension associated with stress. Thus the combination of obesity with chronic stress may underlie the epidemic of cardiovascular disease associated with the adoption of a western lifestyle. In this study we will repeatedly expose mice fed either a normal or high fat diet to a stress on a daily basis over two weeks and record their blood pressure, heart rate and activity continuously via radiotelemetry and determine the effect stress and diet on cardiovascular parameters.

**Project related methods/skills/technologies:**

- Animal handling and surgery
- Direct measurement of blood pressure in conscious freely moving mice
- Stress tests to measure reactivity to aversive and non-aversive stress
- Immuno-histochemical analysis of brain regions

**References:**

## Project title: Circumventing Impaired Nitric Oxide Function in the Cardiovascular Complications of Diabetes

**Laboratory:** Heart Failure Pharmacology

**Primary Supervisor:** Assoc Prof Rebecca Ritchie

**Contact:** rebecca.ritchie@bakeridi.edu.au 8532 1392

### Research Focus:
Our laboratory seeks to improve the understanding of the causes of heart failure, identify new targets for its precursors and develop new pharmacotherapies for delaying their progression.

### Keywords:
Cardiac function; Coronary vasculature; Diabetes; Nitric Oxide; Nitroxyl; Oxidative stress; Platelets.

### Project description:

#### BACKGROUND:
The global epidemic of diabetes mellitus is imposing an exponential burden on society – not only because of the substantial associated healthcare costs, but also because of the poor health outcomes for those with the condition, particularly as a result of diabetic cardiovascular complications-induced morbidity and mortality. Impaired nitric oxide (NO) signalling is an independent marker of poor prognosis. Defined as a diminution or absence of the protective cardiovascular actions downstream of NO, the phenomenon is particularly manifest in diseased human vasculature, likely as a consequence of elevated oxidative stress (see diagram). Loss of NO responsiveness is particularly debilitating in diabetes, where cardiovascular emergencies (acute myocardial infarction, transient ischaemia, cardiogenic shock) occur more frequently, yet the ability of NO-based pharmacotherapies to target platelet aggregation and vasoconstriction is deficient. We have identified nitroxyl (HNO) as a putative strategy for enhancing NO signalling in the heart and vasculature over the short- and longer-term, which we believe can potentially address this clear area of clinical need.

#### GENERAL HYPOTHESIS:
Diabetes-induced NO resistance in the heart and vasculature is exacerbated by poor glycaemic control, and can be circumvented by HNO.

#### AIMS:
- To determine whether diabetes induces NO resistance in the myocardium (not previously known),
- to investigate the relationship between hyperglycaemia and NO responsiveness in the diabetic heart and vasculature, and to demonstrate that HNO circumvents this impaired NO signalling induced by diabetes.

#### SIGNIFICANCE:
Strategies that circumvent (for management of cardiovascular emergencies) and/or ameliorate (targeting the incidence of these emergencies) impaired NO signalling in the diabetic heart and vasculature will improve prognosis in affected patients, of substantial clinical importance.
**Project related methods/skills/technologies:**

- *in vivo* models of diabetic cardiac disease
- isolated rodent hearts *ex vivo*
- assessment of cardiac and vascular function
- biochemical techniques: Westerns, ROS detection, ELISA, real-time PCR, histology

**References:**


Project title: Annexin-A1 Mimetics: a Novel Therapeutic Approach for Targeting the Cardiac Complications of Diabetes

Laboratory: Heart Failure Pharmacology

Primary Supervisor: Assoc Prof Rebecca Ritchie

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Research Focus:
Our laboratory seeks to improve the understanding of the causes of heart failure, identify new targets for its precursors and develop new pharmacotherapies for delaying their progression.

Keywords: Annexin-A1; Cardiac function; Diabetes; Heart failure; Inflammation.

Project description:
BACKGROUND: Diabetes is Australia’s fastest growing chronic disease. The disease affects almost 2 million Australians; diabetes increases heart failure risk 2.5-fold and accelerates its onset. Our laboratory has an established track record for identifying new pharmacotherapies for diabetic cardiomyopathy. Building on this experience, we have obtained recent evidence that cardiac inflammation is a key contributor to myocardial damage in the diabetic heart. We have previously shown that the glucocorticoid-regulated anti-inflammatory mediator annexin-A1 is a key regulator of cardiac viability and function. Annexin-A1 thus offers an attractive approach to minimise the detrimental consequences of diabetes on the heart.

GENERAL HYPOTHESIS: Annexin-A1-based therapies limit diabetic cardiomyopathy by reducing cardiac inflammation and protecting cardiac contractile function.

AIMS: To compare the time-course of cardiac inflammation and impaired cardiac function, in both type 1 and type 2 diabetes, and to investigate annexin-A1 cardioprotection for the cardiac complications of the disease in vivo.

SIGNIFICANCE: The alarming global epidemic of diabetes gives rise to an ever-increasing heart failure burden. We propose that annexin-A1 addresses the unmet clinical need of the extra burden posed by concomitant diabetes, inflammation and cardiomyopathy. The potential cardioprotective annexin-A1 mechanisms represent a strategic new therapeutic intervention for diabetic cardiomyopathy. These interventions may ultimately limit progression to heart failure and death in diabetes-affected patients.
**Project related methods/skills/technologies:**

- *in vivo* models of diabetic cardiac disease
- assessment of cardiac function
- biochemical techniques: Westerns, ELISA, real-time PCR, histology, immunofluorescence

**References:**


**Project title:** Therapeutic targeting of the cardiac hexosamine biosynthesis - ROS axis to protect the diabetic heart

**Laboratory:** Heart Failure Pharmacology

**Primary Supervisor:** Assoc Prof Rebecca Ritchie

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**Research Focus:**
Our laboratory seeks to improve the understanding of the causes of heart failure, identify new targets for its precursors and develop new pharmacotherapies for delaying their progression.

**Keywords:** Cardiac function; Diabetes; Glucose metabolism; Heart failure; Oxidative stress; Mitochondria.

**Project description:**

**BACKGROUND:** Diabetes is Australia’s fastest growing chronic disease. The disease affects almost 2 million Australians; diabetes increases heart failure risk 2.5-fold and accelerates its onset. Our laboratory has an established track record for identifying new pharmacotherapies for diabetic cardiomyopathy, many of which target reactive oxygen species (ROS). Building on this experience, we have obtained recent evidence that the hexosamine biosynthesis pathway (HBP), an alternative fate of glucose, has now emerged as a contributing factor to the cardiac complications of diabetes.

**GENERAL HYPOTHESIS:** that the concomitant impairments in both glucose handling and ROS that are hallmarks of diabetes together provide an additional drive towards unchecked HBP dysregulation, such that this post-translational modification switches from serving an adaptive, to a maladaptive, role, affecting both cardiac function in vivo and mitochondrial integrity.

**AIMS:** The major goal of this study is to demonstrate that cardiac-directed therapeutic targeting of the ROS-HBP axis will delay or even overcome diabetes-induced cardiac dysfunction in the intact heart in vivo.

**SIGNIFICANCE:** The lack of existing management of heart failure in the context of the poorer prognosis of concomitant diabetes highlights a clear, unmet clinical need. By specifically regulating cardiac HBP signalling in either the early or later stages of diabetes-induced heart failure, our approach is therapeutic rather than prophylactic, in addition to permitting tissue-selective regulation of this important, otherwise cytoprotective, post-translational modification.
Project related methods/skills/technologies:

- *in vivo* models of diabetic cardiac disease
- assessment of cardiac function
- assessment of mitochondrial function
- biochemical techniques: Westerns, ELISA, real-time PCR, histology, immunofluorescence

References:


Project title: Targeting the anti-inflammatory protein Annexin-A1 for protection from myocardial infarction (heart attack)

Laboratory: Heart Failure Pharmacology

Primary Supervisor(s): Chengxue Helena Qin & Assoc Prof Rebecca Ritchie

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Research Focus:
Our laboratory seeks to improve the understanding of the causes of heart failure, identify new targets and develop new pharmacotherapies for delaying their progression.

Keywords: myocardial infarction; Annexin A1; anti-inflammation; cardioprotective

Project description:
BACKGROUND: Myocardial ischaemia, in which coronary blood flow is reduced, causes anginal chest pain, myocardial infarction (MI, also known as heart attack), and death. Myocardial infarction represents the major cause of death in Western societies, and in the next decade, this will expand to all corners of the world. The primary determinant of outcome from MI is the extent of cell death during and after ischaemia, from necrosis, apoptosis and/or autophagy. Restoration of blood flow (reperfusion) however is associated with the development of further cell death and impaired recovery of cardiac function, referred to as “reperfusion injury”. Myocardial ischaemia-reperfusion induces an inflammatory response, with damage resulting from both infiltration of circulating inflammatory cells, as well as neutrophil-independent direct actions on myocardium and endothelium (including Ca$^{2+}$ overload, ROS generation and impaired mitochondrial regulation all contributing mechanisms to cell death). In addition, there is incomplete recovery of LV function. Together these phenomena contribute to increased risk of ischaemic cardiomyopathy, heart failure and death. Novel treatment strategies that protect against multiple mechanisms of MI injury will have major clinical impact.

The therapeutic potential of the anti-inflammatory mediator annexin-A1 (ANX-A1), which is the endogenous ligand for formyl peptide receptors (FPR), has been recognized in a range of systemic inflammatory disorders. Importantly, we have shown that ANX-A1 has powerful protective actions against cardiac injury and loss of LV contractile function.

GENERAL HYPOTHESIS: The project will test the hypothesis that ANX-A1 represents a novel modulator of myocardial viability and LV contractile function following ischaemia-reperfusion.

AIM: To investigate the cardioprotective function of endogenous ANX-A1 in ischaemia reperfusion injury, the receptors responsible for cardioprotection elicited by ANX-A1 and its mimetics, and examine the potential therapeutic opportunities offered by exogenous ANX-A1 mimetics after I-R injury in the intact heart.

SIGNIFICANCE: These studies will provide insight into ANX-A1-mediated rescue of myocardial viability and function after I-R injury in the intact heart, and the mechanisms involved. Development of therapeutic strategies for treating myocardial infarction after an unplanned ischaemic event (while reperfusion injury is still evolving), alone or concurrent with standard care, will ultimately reduce progression to heart failure and death in affected patients.
Project related methods/skills/technologies:

- Cell culture (cardiomyocyte)
- Isolated rodent hearts
- Biochemical (Westerns, ROS detection, ELISA, real-time PCR)
- Histological techniques
- *In vivo* model of myocardial infarction

References:


**Project title: A vaccine against atherosclerosis**

**Laboratory:** Biochemistry of Diabetic Complications

**Primary Supervisor (s)**: Chris Tikellis, Merlin Thomas

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**Research Focus:**
Diabetic Complications, Atherosclerosis

**Keywords**
Atherosclerosis, atherogenesis, vaccines

**Project description:**

Atherogenesis is a complex process where a combination of pathogenic factors activates common pathways leading to the development of plaques that progressively narrow and harden our major arteries. The immune system, in particular T-cells, have been shown to contribute to atherosclerosis. Cytotoxic T-Lymphocyte Antigen (CTLA)-4 has an integral role in controlling T-cell activation in atherosclerosis.

CTLA-4 is normally expressed as a homodimer on the surface of T-cells, where it competes with CD28 for binding to the B7 family of co-stimulation molecules, which are expressed on APCs. CTLA-4 allows the T-cell to bind to the antigen presenting cell (APC) resulting in the initiation of an immune response. Blocking CTLA-4 can reduce atherosclerotic plaque lesions in a mouse model of atherosclerosis. The problem with current inhibitor strategies is that most approaches use a monoclonal antibody against CTLA-4. Consequently, after a few weeks the host raises an immune response against the CTLA-4 antibody.

This project will use a new approach that will mimic a vaccine except in this case against atherosclerosis and not a conventional pathogen. We have constructed a CTLA-4-measles fusion protein that may be used as a DNA vaccine to deliver the protein in vivo. The theory behind this approach is that the CTLA-4 protein will prevent systemic T-cell activation by blocking the APCs from binding and activating T-cells. The measles component of the protein will generate an immune response allowing the delivery efficacy of the vaccine to be evaluated.

The project offered will involve animal work, primary cell cultures and evaluation of immunological markers. The student will be part of a research team and will learn a number of techniques throughout their project that will include atherosclerotic plaque area quantitation, RNA extraction, cDNA synthesis, Real Time PCR, ELispots and ELISAs.

**Project related methods/skills/technologies:**
- RNA extraction
- cDNA synthesis
- RT-PCR
- Elispot and ELISAs
- Animal work
- Primary Cell cultures
**Project title:** Metabolic memory - the bitter legacy of high glucose levels

**Laboratory:** Biochemistry of Diabetic Complications

**Primary Supervisor (s)** Chris Tikellis, Merlin Thomas

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**Research Focus:**
Diabetic Complications, Atherosclerosis, Glucose control

**Keywords**
Metabolic memory, Diabetes, hyperglycaemia

**Project description:**

Metabolic memory is the name given to the phenomenon whereby previous exposure to metabolic perturbations (such as high blood glucose levels) has long-lasting physiological effects, long after the event has dissipated. For example, a period of suboptimal blood glucose control in patients with diabetes, continues to be a risk factor for adverse outcomes, when compared to those patients who were initially intensively treated, despite the fact that glucose control has been subsequently identical in both groups of patients for over a decade.

We have shown in animal models of diabetes, that restoration of healthy glucose control does not reduce atherosclerosis and the pro-inflammatory impact of hyperglycaemia when compared to that seen in mice with persistent hyperglycaemia. It is said the sweetest things are hardest to forget. However, the scientific question of how periods of poor glucose control can have persistent effects, even decades later, is pivotal to our approach to managing diabetes. It is also apparent that even transient elevations in blood glucose may be sufficient to initiate a range of pathogenic pathways associated with an increased risk of micro and macrovascular damage, even while mean glycaemic control may be maintained within normal range.

The project offered will involve animal work, primary cell cultures and epigenetic profiling. The animal work will consist of studies where mice are exposed to high glucose levels to achieve a transient elevation in blood glucose levels. Aortas from these mice will be used for gene expression, protein quantitation and for the isolation of primary endothelial cells.

The student will be part of a research team and will learn a number of techniques throughout their project that will include using an animal model of transient hyperglycemia, RNA extraction, cDNA synthesis, Real Time PCR, ELISAs, tissue culture, epigenetic profiling and aortic endothelial cell isolation.

**Project related methods/skills/technologies:**
- RNA extraction
- cDNA synthesis
- RT-PCR
- ELISAs
- Tissue Culture
- Epigenetic profiling
- Endothelial cell isolation
**Project title:** The role of Angiotensin 2 receptor (AT2R) in atherosclerosis

**Laboratory:** Diabetes and Atherosclerosis

**Primary Supervisor(s):** Terri Allen and Christine Koulis

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**Research Focus:** Diabetes, Angiotensin, AT2R, signaling pathways

**Keywords:** atherosclerosis, diabetes, oxidative stress

**Project description:**
The renin-angiotensin system (RAS) contributes to the elevated risk of CVD, including atherosclerosis, in diabetics. While much is known about the AT1R in the context of diabetes, little is known about the other AngII receptor subtype, AT2R. The AT2R has been presumed to act as a physiological antagonist to the AT1R. However, the role of the AT2R in different physiological settings is still unclear. Elucidating the actions of the AT2R in physiological and pathological states will help in defining the potential importance of the AT2R in therapeutic development.

We showed that the AT2R is expressed in the atherosclerotic aorta and is upregulated in the diabetic setting at the gene level and reduced with AT2 gene deletion (DK) or pharmacological inhibition (PD). We compared equally hyperlipidaemic atherosclerotic diabetic and high fat fed mice, however, aortic AT2R upregulation was only observed in diabetic mice in association with prominent macrophage infiltration - a feature of DAA. Furthermore, we showed a ten-fold increased expression of the AT2R in blood mononuclear cells (PBMC) from diabetic apoE KO mice. PBMC AT2 expression is reduced with PD123319 treatment and is absent in the AT2R/ApoE double KO (DKO) mouse.

Pro-atherosclerotic effects of the AT2 receptor in vivo: In recent studies, plaque formation in diabetic apoE KO mice was decreased by ~50% in mice treated with the AT2R antagonist, PD123319, for 20-weeks. Furthermore, diabetic AT2R/apoE DKO mice had ~50% reduction in plaque area when compared to diabetic apoE KO mice (D) which was associated with reduced inflammation. Based on these data, we suggest that the AT2R upregulation is not purely a manifestation of atherosclerosis but represents a pathogenic receptor mediated pathway leading to atherosclerosis.

**RESEARCH PLAN**
To further understand the involvement of C21 in potentiating atherosclerosis we would like to employ two different strategies to inhibit the involvement of the AT2R: genetic deletion and pharmacological antagonism.

**AIMS**
The general aim of the present study is to validate and elucidate the mechanism of the pro- and anti-atherosclerotic effects of AT2R activation, with C21, in DAA.

1. To assess DAA in an AT2R KO mouse (ApoE-/-:AT2R-/-) with and without C21 at LD and HD.
2. To assess DAA in an ApoE KO mouse with and without C21 at LD and HD and in the presence and absence of a selective AT2R antagonist, PD123319.

Aim 1. Diabetes is induced in ApoE mice at 6-weeks of age and followed for 20-weeks. Diabetes will be induced by injection of streptozotocin (55 mg/kg) intraperitoneally, daily for 5 days. The control group will receive citrate buffer alone. Only mice with a blood glucose >15 mmol/l after the 6th day will be included in the study. After induction of diabetes, animals will be randomised to receive control or treatment for 20-weeks. Compound 21, will be administered at two different doses, low dose (LD; 0.1mg/kg/day via gavage) and high dose (HD; 10mg/kg/day via gavage).
Aim 2. Investigate the effect of the PD123319 on DAA in mice treated with C21. The protocol is as described in Aim 1, however, the stain and treatments are as indicated in the table below. PD123319 will be administered by Alzet osmotic mini pump subcutaneously and replaced every 4 weeks at a dose of 5mg/kg/d.

**END POINT PARAMETERS**

Atherosclerotic plaque area: After 20 weeks of study, the aorta will be stored in formalin or snap frozen. The processed aortae will be used for measurement of atherosclerotic lesion area (en face and aortic sinus), tissue sectioning and subsequent immunohistochemical studies. The frozen aortae will be used either to generate mRNA for real time RT-PCR or for evaluation of proteins as assessed by immunohistochemistry.

Cellular/molecular markers to be assessed:
- Inflammation: macrophages and VCAM-1 by immunohistochemistry
- RAS: AT1R and AT2R by RT-PCR
- Chemokines: MCP-1 by RT-PCR
- Oxidative stress: Hydrogen peroxide, superoxide, nitrotyrosine, gp91phox, gp47phox, rac-1, p22phox by RT-PCR and immunohistochemistry.

**Project related methods/skills/technologies:**
- Immunohistochemistry
- RT-PCR
- Animal models
**Project title:** Antioxidant nanozymes and their role in endothelial dysfunction.

**Laboratory:**
Oxidative Stress Laboratory, Diabetic Complications, Baker IDI Heart and Diabetes Institute.

**Primary Supervisor(s):** Dr Judy B. de Haan

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**Research Focus:** Novel targeted antioxidant treatments to improve NO bioavailability and improve diabetic endothelial dysfunction.

**Keywords:** Endothelial dysfunction, oxidative stress, vascular injury, type 1 and type 2 diabetes, NO bioavailability, antioxidant nanoparticles.

**Project description:** Diabetes and cardiovascular disease (CVD) remain the leading cause of morbidity and mortality in Western societies. Diabetes, initially thought of as a disease of impaired glucose metabolism, is now considered an independent risk factor for CVD with approximately 70% of deaths occurring as a result of a vascular event. The mechanism by which diabetes dramatically increases the risk of CVD is incompletely understood, but is believed to be initiated by impairment of the vascular endothelium, a condition known as endothelial dysfunction (ED)[1]. Most current theories stipulate that recovery of endothelial function will lead to vascular protection, health and longevity [2]. A major approach to improve endothelial function is to reduce oxidative stress and increase the bioavailability of nitric oxide (NO), a critical mediator of endothelial function. This is particularly relevant in diabetic patients where oxidative stress is elevated and NO synthesis is markedly reduced [3]. Thus, the development of novel targeted strategies to attenuate oxidative stress and restore NO bioavailability should be the primary goal of therapy to improve endothelial dysfunction.

This project will investigate the use of novel antioxidant approaches to lower oxidative stress, restore NO bioavailability and thereby improve endothelial dysfunction associated with diabetes. In particular novel nanoparticles will be tested in cell culture (human aortic endothelial cells) and in animal models of diabetes. Cell lines will be used to investigate signalling mechanisms involved in these processes.

Since macrophages play a prominent role in inflammatory processes that drive ED by releasing cytokines and ROS, the student will also investigate whether our novel antioxidant approaches lessen oxidative stress and thereby cytokine release, two prominent factors that play a role in macrophage signalling that drives ED in diabetes.

Several projects (suitable for both Honours and Ph.D.) are available to study the effects of these novel compounds on improving endothelial dysfunction associated with diabetes.

**Project related methods/skills/technologies:**

The project will train the student in **various techniques** including working with diabetic animals, isolation of primary macrophages, assessment of endothelial function both in cell lines and ex-vivo, measures of oxidative stress, the expression of inflammatory factors by qRT-PCR, immunohistochemistry for vascular inflammatory markers, and assessment of leukocyte adhesion both in cultured cells and in isolated aortas. Other techniques will include western blotting, genotyping, and confocal microscopy.

**References:**

**Project title:** Novel antioxidant treatments to limit glucose-driven retinal vascular injury

**Laboratory:**
Oxidative Stress Laboratory, Diabetic Complications, Baker IDI Heart and Diabetes Institute and Dept Immunology, Monash University.

**Primary Supervisor(s):** Dr Judy B. de Haan and Prof Jenny Wilkinson-Berka

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AND
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**Research Focus:** Novel targeted antioxidant treatments to lessen vascular injury associated with diabetic retinopathy.

**Keywords:** Diabetic Retinopathy, oxidative stress, microvascular injury, type 1 and type 2 diabetes.

**Project description:**
Vascular complications are a major health burden associated with diabetes. Micro- and macrovascular complications including kidney disease, eye disease and heart disease are on the increase given the dramatic rise in the incidence of diabetes worldwide. The mechanisms that drive this acceleration in diabetic vascular disease are still not completely understood. There is increasing evidence that oxidative stress, mediated by the increase in glucose, plays a significant role particularly in the retina of the eye where lipids are particularly susceptible to oxidative injury.[1] Diabetic retinopathy (DR) is a major cause of blindness in individuals with both type 1 and type 2 diabetes despite tight glycaemia control and the use of anti-hypertensive therapy.

Therefore, there is an urgent clinical need to develop new therapies to reduce this significant health burden.

This project will investigate the use of novel antioxidant approaches to lessen vascular injury associated with DR in cell culture and animal models. Our strong preliminary data suggest that bolstering antioxidant defences will lessen the oxidative burden associated with diabetes, positively impact on pro-inflammatory pathways and reduce structural and functional deficits of DR in our models. We now have access to novel compounds, including activators of the transcription factor Nrf2 and antioxidant-like nanoparticles with which to explore whether our approach of bolstering antioxidant defences can prevent the progression of DR in two well-established DR models. Our models include the diabetic Akita mouse and a model of oxygen-induced retinopathy.[2] Animal studies will be complemented by in vitro studies in cells treated with high glucose as well as cells obtained from diabetic animals. Cell lines will be used to investigate signalling mechanisms involved in these processes.

Several projects (suitable for both Honours and Ph.D.) are available to study the effects of these novel compounds in DR with the potential to translate these pre-clinical findings into clinical treatments for DR.

**Project related methods/skills/technologies:**
The project will train the student in various techniques including working with diabetic animals, assessment of retinal damage including vascular leakage, leukostasis, assessment of microglial density, gliosis, cellular apoptosis, oxidative stress measurements, immunohistochemistry for retinal gliosis, and the expression of angiogenic and inflammatory factors by qRT-PCR. Other techniques will include western blotting, genotyping, as well as confocal microscopy, and in vitro cell culture experiments.

**References:**
**Project title:** The role of intrarenal nerves in the diabetic and hypertensive kidney

**Laboratory:** Diabetic Complications

**Primary Supervisor (s):** Dr Anna Watson & Prof Karin Jandeleit-Dahm

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**Research Focus:** Kidney disease

**Keywords**
Diabetic Complications, Hypertension, Neurobiology, Kidney Disease

**Project description:**

With the increasing prevalence of both type 1 and 2 diabetes, new treatments are urgently needed to reduce the burden for people developing diabetic complications, especially diabetic kidney disease. Hypertensive individuals often have diabetes as a co-morbidity and develop kidney disease at an accelerated rate compared to people without hypertension. A controversial new therapeutic treatment in which the renal nerves are severed (ablated) holds great promise for reducing blood pressure in patients that are unresponsive to drug treatments. We have evidence to suggest that the renal nerves are altered in both diabetes and hypertension, however how these changes effect the development of diabetic or hypertensive kidney disease is unknown. Similarly, what effect severing renal nerves has upon the kidney in the long term is not known.

The project will involve investigating changes in nerves within the diabetic and hypertensive kidney utilizing various techniques including immunohistochemistry, RT-PCR for gene expression analysis and cell culture. These technologies will be combined to demonstrate how renal nerves contribute to development of kidney disease in hypertension and diabetes, and how the therapeutic intervention of severing the nerves to the kidney affects this.

**Project related methods/skills/technologies:**

- Cell culture
- RT-PCR
- Western blot/ELISA
- Tissue morphology & histology

**References:**

**Project title:** The role of Nox5 in diabetic vascular and renal disease

**Laboratory:** Diabetic Complications

**Primary Supervisor(s)** Dr. Stephen Gray, Prof. Karin Jandeleit-Dahm & Mr. Jay Jha

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**Research Focus:**  
Oxidative Stress, Diabetic Complications, Vascular and Kidney Disease

**Keywords**

**Project description:**

The development of diabetic complications including vascular and renal disease is enhanced in diabetic patients. However, the underlying mechanism as to why this disease process is accelerated is largely unknown.

Oxidative stress has been proposed to play a key role in the development of diabetic complications, particular the NADPH oxidase (Nox) family. There are primarily 4 Nox isoforms that have been shown to be important in the development of diabetic complications, Nox1, 2, 4 and 5. All of these isoforms have increased expression and activity in diabetic patients. Our group has established a role for the Nox1 isoform in the development of diabetic atherosclerosis, and the Nox4 isoform in diabetic kidney disease. However, to date a role for the Nox5 isoform in diabetic complications is unknown.

**We have multiple honors projects involving the role of Nox5 in diabetic complications, which will focus on the following areas:**

1. **Vascular Disease** (Dr. Stephen Gray, Prof. Karin Jandeleit-Dahm)
2. **Kidney Disease** (Dr. Stephen Gray, Mr. Jay Jha)

The projects will utilize a highly novel humanised Nox5 knockin mouse, where Nox5 is expressed only in the vascular smooth muscle cells or the endothelial cells. The project will involve applying various experimental techniques that include immunhistochemistry, RT-PCR for gene expression analysis and cell culture analysis to delineate the role that Nox5 plays in the development of diabetic complications.

**Project related methods/skills/technologies:**

- Cell Culture
- RT-PCR
- Tissue morphology & histology

**References:**

1. Gray SP., *et al.*  Nox1 plays a key role in diabetes associated atherosclerosis *Circulation.* 2013
Project title: Targeting the C5a-CD88 axis in diabetic nephropathy

Laboratory: Glycation, Nutrition & Metabolism

Primary Supervisor(s): A/Prof Melinda Coughlan, Dr Sih Min Tan

Contact: Melinda.Coughlan@bakeridi.edu.au 8532 1278

Research Focus:
Inhibition of a key pathway of inflammation in the progression of diabetic kidney disease

Keywords: Kidney Disease, Diabetes, Inflammation, Pre-clinical studies, Human Translation.

Project description:
Background: Over 400,000 Australians are affected by diabetic kidney disease. Current clinical therapies only delay the progression to end stage renal disease and thus new therapies are urgently required. The main aim of our group is to understand why people develop complications from diabetes, and the mechanisms responsible for those complications, in the hope of finding new therapeutic targets that can halt progression to end stage renal disease.

Activation of the complement system is a major pathogenic event that drives various inflammatory responses in numerous diseases. The “anaphylatoxin” complement C5a is a major effector of all complement activation pathways (classical, mannose binding lectin and alternative) and a potent pro-inflammatory mediator. Previous studies have suggested that the complement system may be involved in the pathophysiology of diabetic nephropathy, though the role of specific complement pathways in the development of this disease remains incompletely defined.

Research question: Is the C5a receptor a therapeutic target for diabetic nephropathy?

Aim 1. To characterise complement function and activation during the development and progression of diabetic kidney disease
Aim 2. To determine if targeting the pro-inflammatory complement receptor C5aR/CD88 prevents the development and/or progression of diabetic nephropathy.

We have a C5a receptor antagonist that will be trialed in a mouse model of type 1 diabetes (the Ins2-Akita mouse). The development of diabetic nephropathy will be assessed using immunohistochemistry of renal cortex to assess renal pathology, renal functional measures, and markers of fibrosis, reactive oxygen species and inflammation. Proteins of the complement pathway will be determined.

In order to translate our findings from mouse models to human disease, we will characterise complement activation products and receptor expression in patients with diabetes and renal disease. In collaboration with the Austin Hospital we have access to plasma, urine and renal biopsy samples from patients with diabetes which will be used to assess complement activation products and receptor expression.

Opportunities exist for Honours, Masters and PhD students.

Project related methods/skills/technologies:

- Mouse models of diabetes/pre-clinical studies.
- Mouse metabolic phenotyping and body composition.
- Western immunoblotting and ELISA.
- Fluorogenic and colorimetric enzyme activity assays.
- Renal histology to determine pathology.
## Project Title: The effect of processed foods on gut homeostasis and kidney disease

### Laboratory:
Glycation, Nutrition & Metabolism

### Primary Supervisor(s):
A/Prof Melinda Coughlan

### Contact:
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### Research Focus:
Investigating the effects of processed foods (Advanced Glycation End Products) on gut homeostasis and disease pathogenesis.

### Keywords:
Nutrition, Gut Homeostasis, Kidney Disease

## Project Description:

### Background:
Advanced glycation endproducts (AGEs), formed via the processing of foods, are a major constituent of our modern convenience diet. Environmental factors, including over-nutrition and excess dietary intake of AGEs are suggested to contribute to the progression of chronic diseases, including diabetes and chronic kidney disease (Coughlan et al., Diabetes 2011) and studies from our laboratory suggest that dietary AGEs may affect the gut (Forbes et al., J Nutr Biochem 2013). Microbial cells make up the majority of cells in the human body, and most of these reside in the intestinal tract. Emerging evidence suggests that bacterial dysbiosis within the colon may be implicated in the pathogenesis of the metabolic syndrome, type 2 diabetes and cardiovascular disease. How dietary AGEs influence the gut microbiota and function is not known.

### Research Question:
Does a high AGE diet influence gut homeostasis, leading to disease?

### Aim:
The aim of this study is to investigate the effects of excess consumption of dietary AGEs on gut microbiota and homeostasis, and the progression of chronic kidney disease in a mouse model, with or without prebiotic supplementation.

### Opportunities exist for Honours, Masters and PhD students.

### Project related methods/skills/technologies:
- Dietary intervention studies.
- Mouse metabolic phenotyping and body composition.
- Western immunoblotting and ELISA.
- Fluorogenic and colorimetric enzyme activity assays.
- Renal and gut histology to determine pathology.

### References:


Forbes JM, Cowan SP, Andrikopoulos S, Morley AL, Ward LC, Walker KZ, Cooper ME, Coughlan MT. Glucose homeostasis can be differentially modulated by varying individual components of a western diet. Journal of Nutritional Biochemistry 2013 24(7): 1251-1257
**Project title:** Targeting dysfunctional mitochondria by mitophagy in diabetic nephropathy

**Laboratory:** Glycation, Nutrition & Metabolism

**Primary Supervisor(s):** A/Prof Melinda Coughlan, Dr Gavin Higgins

**Contact:** Melinda.Coughlan@bakeridi.edu.au 8532 1278

**Research Focus:** Investigating mitochondrial dysfunction in diabetic kidney disease

**Keywords:** Kidney Disease, Diabetes, Mitochondria, Mitophagy

**Project description:**

**Background:** Diabetic nephropathy (DN) is a progressive microvascular complication arising from diabetes. Within the kidney, the glomeruli, tubules, vessels and interstitium are disrupted, ultimately impairing renal function, and leading to end stage renal disease (ESRD) and death. Current therapies used in individuals with DN do not prevent the inevitable progression to ESRD, therefore new targets of therapy are urgently required. Studies from animal models indicate that disturbances in mitochondrial homeostasis are central to the pathogenesis of DN. Accumulation of damaged mitochondria are found in the renal cortex in animals with DN, suggesting that mitochondrial clearance mechanisms may be impaired. In order to maintain a pool of functional mitochondria, cells have a housekeeping process called autophagy, which selectively removes damaged proteins and organelles. The process of removing damaged mitochondria is termed mitophagy. In diabetic nephropathy the accumulation of damaged mitochondria is thought to contribute to its pathology. The aim of this project will be to further understand how mitophagy is activated during the early stages of diabetic nephropathy and why this process is unable to continue clearing damaged mitochondria late in diabetes.

**Research question:** Does mitophagy impairment contribute to diabetic nephropathy pathology?

**Aim:** To identify the mechanisms that regulates mitophagy activity in human proximal tubule epithelial cells (PTECs) and to establish their role in DN.

To better understand mitophagy under diabetic conditions this project will involve culturing human PTECs and exposing them to high glucose, as well as pharmacological inhibitors of autophagy and siRNA to silence autophagic genes.

**Opportunities exist for Honours, Masters and PhD students.**

**Project related methods/skills/technologies:**

- Western immunoblotting and ELISA.
- Fluorogenic and colorimetric enzyme activity assays.
- Renal histology to determine pathology.
- Confocal microscopy
- Cell culturing of human PTECs.
- Assessment of mitochondrial morphology (using electron microscopy) and bioenergetics (using the Seahorse bioanalyser).

**References:**

**Project title:** Investigating the interactions between RAGE and GLP-1 in diabetes and diabetic nephropathy

**Laboratory:** Glycation Nutrition and Metabolism Laboratory

**Primary Supervisor(s):** Dr. Karly Sourris and Dr. Melinda Coughlan

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**Research Focus:**
Keywords: Diabetes, Glycation, Diabetic Nephropathy

**Project description:**
It is estimated by 2020 some 180 million people worldwide will be diagnosed with diabetes. Up to 40% of individuals with diabetes develop serious complications including kidney disease, which is a major cost to Australia’s healthcare system. In diabetes, present therapies only slow down the progression of kidney disease. Therefore, the focus of our group is to elucidate the mechanisms involved in the development and progression of diabetic kidney disease. In addition, the identification of novel therapeutic targets will ultimately improve current treatment approaches. It is for this reason that we are investigating the interactions between a protein which is part of our body’s defence system, the receptor for advanced glycation end products (RAGE) and glucagon-like peptide (GLP-1), a modulator of blood glucose in the diabetic kidney. Ultimately, it is anticipated that the findings of the project will assist in a more rational design of future therapies to combat the development and progression of diabetic kidney disease. This project will measure diabetic, renal function and oxidative stress parameters in control and diabetic c57BL/6, RAGE and GLP-1 knockout mice. In addition, we will be translating our findings to the clinical setting by investigating this interaction in clinical samples collected from both type 1 and type 2 diabetic patients.

**Project related methods/skills/technologies:**
- Cell Culture
- Western Blotting
- Enzyme-linked Immunoabsorbent Assay (ELISA)
- Tissue Homogenisation
- Flow Cytometry
Project title: IDOL-mediated regulation of lipid metabolism in skeletal muscle and the heart

Laboratory: Diabetes & Dyslipidaemia

Primary Supervisor(s): Brian Drew & Anna Calkin

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Research Focus: lipid metabolism, E3 ligase, diabetes, cholesterol, triglycerides

Project description:

Individuals with diabetes are 3-4 times more likely to develop cardiovascular disease including stroke and heart attack. One of the most common causes of cardiovascular disease is dyslipidaemia. Indeed, individuals with diabetes commonly have elevated levels of triglycerides or LDL cholesterol, and reduced HDL cholesterol levels, each an independent risk factor for cardiovascular disease. The excess cholesterol and triglycerides can be deposited in tissues such as the heart, liver and skeletal muscle where they have detrimental effects, promoting atherosclerosis, insulin resistance and cardiac dysfunction. If we can prevent these excess lipids from entering these tissues, then we can prevent the abovementioned pathological effects, seen particularly in the setting of diabetes.

IDOL, or inducible degrader of the LDL receptor, is a recently identified E3 ligase (1). It acts in a “substrate recognition” capacity to identify and ubiquitinate its targets in preparation for their degradation. IDOL has three targets, the LDL receptor (LDLR), the VLDL receptor (VLDLR) and the apoE receptor (ApoER2), which are involved in the uptake of lipids. Studies have shown that IDOL is important for the regulation of plasma cholesterol levels in humans (2).

This project will examine whether IDOL can reduce the accumulation of cholesterol or triglycerides in peripheral tissues including the heart and skeletal muscle, and in turn prevent skeletal muscle insulin resistance and cardiac dysfunction.

Project related methods/skills/technologies:

- Protein and RNA extraction, Western Blotting, qPCR
- Cell culture, knockdown and over-expression studies
- AAV mouse models, tissue dissection and analysis

References:

**Project title:** A novel mechanism to promote mitochondrial health and prevent skeletal muscle insulin resistance and diabetes.

**Laboratory:** Diabetes & Dyslipidaemia

**Primary Supervisor (s):** Brian Drew & Anna Calkin

**Contact:** Brian.drew@bakeridi.edu.au

**Research Focus:**
Skeletal muscle, diabetes, metabolism, mitochondrial health

**Project description:**
The amount of HSP72 protein found in muscle, significantly decreases when a person has diabetes. Studies show that increasing the amount of HSP72 back to normal, using drugs or gene therapy, can prevent the onset of obesity and diabetes. We recently described in two separate publications that this protective effect is due in part to HSP72 maintaining the health of mitochondria (the cells power-stations) in skeletal muscle, thus protecting the muscle from insulin resistance. Mechanistically, HSP72 achieves this through direct binding and modulation of the activity of an enzyme called Parkin, a key switch in cells that regulates targeted removal of damaged mitochondria. Retention of damaged mitochondria has long been associated with diabetes and insulin resistance. These findings suggest that HSP72 may protect from obesity and diabetes by improving the cells ability to get rid of damaged mitochondria. Given that drugs which increase the levels of HSP72 are already available for human use, it will be important to know whether these drugs improve the clearance of damaged mitochondria and therefore prevent obesity and diabetes.

**HYPOTHESIS & AIMS:** We hypothesize that HSP72 and Parkin interact via specific sites within both proteins, and that particular cellular insults are necessary to induce the interaction and promote targeted degradation of damaged mitochondria in skeletal muscle. We will test this hypothesis with the following aims:

1. **Is the interaction between HSP72 and Parkin direct and what motifs and other proteins are important for complex formation?**
2. **What cellular insults promote interaction of HSP72 and Parkin, and what is the mechanism?**
3. **Can genetic or pharmacological manipulation promote the interaction between HSP72 and Parkin in skeletal muscle and thus improve mitochondrial activity and energy metabolism?**


**Project related methods/skills/technologies:**
- Muscle physiology and metabolism
- Protein expression, binding assays, immunoprecipitation and mutagenesis
- Cell culture, Western blotting, quantitative PCR,
- Knock-out and transgenic mouse models

**References:**
3. Henstridge, D. C.; Activating HSP72 in rodent skeletal muscle increases mitochondrial number and oxidative capacity and decreases insulin resistance *Diabetes* (2014)
Project title: A Novel Enzyme with Potential in Preventing Obesity induced Diabetes.

Laboratory: Diabetes & Dyslipidaemia

Primary Supervisor (s): Brian Drew & Anna Calkin

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Research Focus:
Obesity, adipose tissue, fat storage, metabolism, diabetes

Project description:
Obesity promotes health complications including skeletal muscle insulin resistance (pre-diabetes) and liver disease. One of the primary reasons that obesity promotes these diseases is because of the unwanted storage of excess fat (lipid) - that can no longer go to fat (adipose) tissue where it belongs - in tissues such as muscle and liver. As such, prevention of obesity related diseases may in part rely on reducing the deposition of lipid in these tissues. This is important, because obesity per se is difficult to treat and current therapies mostly rely on minimizing the impact of the disease by stopping the uptake of lipid in the intestine (by medication), or promoting utilization of stored lipid (exercise). These current therapies however are clearly not sufficient, as rates of obesity still continue to rise.

In collaboration with the genetics department at UCLA, our lab has used high-resolution gene mapping to identify a new enzyme that is found in high amounts in adipose tissue and whose expression level is significantly dependent on the level of obesity in both rodents and humans. Preliminary data suggests that this enzyme is regulated by adipose specific factors, and mechanistically acts to inhibit the maturation of adipose tissue. Thus, it is hypothesised that during obesity, inhibition of this enzyme should promote positive adipose tissue expansion, providing a greater capacity to store lipid in adipose tissue and therefore reduce lipid deposition in muscle and liver, preventing disease.

Experimental outline:
Initial studies will be cell culture based, aimed at identifying the targets of this enzyme and confirming its role in regulating fat-cell (adipocyte) function. Longer term studies will include characterizing the adipose-specific knock-out mouse model and determining if viral (AAV) mediated knock-out of the enzyme can prevent diabetes in numerous models of obesity (high fat diet induced and genetic – ob/ob).


Project related methods/skills/technologies:
- Cell culture, Immunoprecipitation and Western blotting
- qPCR, cloning, mutagenesis
- SILAC, mass-spectrometry
- Adeno-associated Virus (AAV) and knock-out mouse models

References:
Project title: Characterisation of the IC7 Transgenic mouse

Laboratory: Cellular and Molecular Metabolism Laboratory

Primary Supervisor(s):
Mark Febbraio and Tamara Allen

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Research Focus:
Treatment of type 2 diabetes

Keywords:
obesity, type 2 diabetes,

Project description:
Our laboratory has been investigating the therapeutic use of IC7 for the treatment of obesity induced type 2 diabetes. IC7 is a mixture of two naturally occurring proteins (interleukin 6 and ciliary neurotrophic factor) that have both been found to improve fat and glucose metabolism\(^1,2\). Treatment of mice with IC7 led to an improvement in fasting blood glucose levels and glucose tolerance as well as weight loss and decreased food intake. To fully investigate the many metabolic effect of IC7, we have generated a mouse that produces IC7, mimicking a treatment regime in mice. This allows us to more easily investigate the metabolic changes that occur with treatment as well as the mechanism by which these changes occur. This project entails feeding the IC7 transgenic mice with a high fat diet and investigating changes in blood glucose levels and the ability of the mice to utilize glucose and fat. Specific tissues will also be analysed to understand the pathways by which these actions take place. This work will allow us to fully understand how IC7 is capable of reducing blood glucose in high fat fed mice and thus continue to develop this protein in the hope of generating a new therapy to treat type 2 diabetes.

Project related methods/skills/technologies:

- Animal handling
- Western blots
- ELISAs
- RNA analysis
- Metabolic caging

References:

Project title: The role of Heat shock protein 25/27 (HSP25/27) in skeletal muscle insulin resistance

Laboratory: Cellular and Molecular Metabolism

Primary Supervisor(s)
Darren Henstridge, Mark Febbraio

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Research Focus:
Study of the effects of Heat shock protein 25/27 on skeletal muscle glucose and fat metabolism in the setting of insulin resistance

Keywords
Heat shock proteins, insulin resistance, skeletal muscle, glucose metabolism, fat metabolism, type 2 diabetes, obesity

Project description:
The prevalence of both obesity and type 2 diabetes is increasing at a dramatic rate both in Australia and worldwide. Obesity is highly associated with a state known as insulin resistance which precedes the onset of type 2 diabetes. Insulin resistance is the physiological condition by which cells in insulin sensitive tissues such as skeletal muscle, adipose tissue and the liver fail to respond to the normal actions of the hormone insulin which leads to high levels of blood glucose (hyperglycemia) and insulin (hyperinsulinemia).

Our laboratory has been working on the protein heat shock protein 72 (Hsp72) for many years. Hsp72 is known as a chaperone protein and works to protect damaged proteins in the cell to maintain normal cellular homeostasis. We have previously shown that patients with type 2 diabetes have lower levels of skeletal muscle Hsp721,2. In animal studies, we have demonstrated that increasing (overexpressing) Hsp72 in skeletal muscle protects mice against the deleterious effects of a high fat diet which causes insulin resistance2,3. Conversely mice that have had Hsp72 deleted from their tissues (HSP72 knockout) are more prone to weight gain and insulin resistance4. We now wish to see if this is also the case for another heat shock protein - Hsp25/27. Hsp25 is the mouse version of the human Hsp27 protein, a member of the small Hsp family comprised of a diverse group of proteins. Interestingly, Hsp25 expression is significantly reduced in both slow-twitch oxidative skeletal muscle (soleus) and mixed muscle (epitrochlearis muscle) with increasing age (3 month old versus 24 month old rats) This drop in Hsp expression corresponded to a decrease in glucose uptake and insulin signaling in the muscle with age5. Thus we now wish to investigate whether Hsp25/27 overexpression improves markers of metabolism.

Finding new therapeutics for obesity and type 2 diabetes is of great importance given the complications that arise from these conditions. Understanding the cellular and molecular mechanisms involved in these conditions may lead to new therapeutic approaches for the future.

Opportunities exist for Honours, Masters and PhD students.

Project related methods/skills/technologies:
*Microbiology – growing DNA plasmids to overexpress HSP25/27
*Cell culture – Growing muscle cells to test the effects of HSP25/27 on muscle glucose uptake, mitochondrial function, fatty acid oxidation & insulin signalling in an in vitro model
*Animal work – Working with mice fed a normal chow diet or a high fat diet and then overexpressing HSP25/27 in a leg skeletal muscle to test the effects on glucose metabolism, insulin signalling and mitochondrial function in an in vivo model
*Laboratory Analysis- Western blotting, qPCR, radiolabelled assays
References:
Project title: Investigating the therapeutic potential of targeting the bone morphogenetic protein signalling pathway to combat skeletal muscle wasting

Laboratory: Muscle Research and Therapeutics

Primary Supervisor(s): Dr Paul Gregorevic

Contact: email: paul.gregorevic@bakeridi.edu.au phone: 8532 - 1224

Research Focus: Developing molecular interventions to dissect mechanisms controlling skeletal muscle attributes and generate gene therapies for conditions of muscle wasting.

Keywords: Skeletal muscle, cell signaling, growth regulation, gene therapy, wasting, cancer cachexia, sarcopenia, muscular dystrophy, aging.

Project description:

Skeletal muscle comprises approximately 40% of our body mass and performs a number of crucial bodily functions. Loss of muscle mass and strength is a serious and unmet health risk associated with disability, illness and premature death, having serious consequences on the vast majority of society.

The Laboratory for Muscle Research and Therapeutics Development aims to identify the molecular mechanisms that control muscle mass and manipulate cellular signalling pathways to promote muscle hypertrophy, metabolic function and strength in disease.

Our lab specialises in the delivery of genes to the striated muscle using recombinant viral vectors. This cutting edge technology allows us to reveal the basic biological characteristics of skeletal muscle in vivo in addition to providing a platform to investigate gene therapies in mouse models of skeletal muscle wasting.

A particularly novel signalling pathway controlling skeletal muscle mass is the bone morphogenetic protein (BMP) signaling pathway which was originally discovered for its role in stimulating bone formation. We have recently shown that this pathway plays a vital role as a positive regulator of skeletal muscle mass. Increasing BMP signaling in muscle can promote muscle growth, and counter protein breakdown. Based on these exciting results, we are now seeking to investigate whether gene-delivery and drug based interventions can be developed which target this pathway, as new therapeutics for muscle wasting.

The central aim of this project will be to use a number of molecular approaches to increase or reduce BMP signalling in skeletal muscle and accompanying cell types in mouse models of disease, to determine if this impacts on the loss of muscle mass associated with muscle wasting conditions, and impacts on lifespan. Additional studies will examine the signaling and gene regulation associated with these effects. Research projects are available for Honours, Masters and PhD students.

![Diagram](Healthy Muscle ➔ Nerve damage ➔ Activation of muscle catabolism ➔ Muscle wasting ➔ Up-regulation of BMPs ➔ Preservation of muscle mass)
Project related methods/skills/technologies:

- Analysing skeletal muscle phenotypes in vivo
- Designing and administering viral vectors in vivo
- Immunofluorescent microscopy
- Cell culture validation of viral vector plasmids
- Using multiple mouse models of skeletal muscle wasting
- Physiological assessment of skeletal muscle function
- Molecular assays including western blotting and q-PCR
- Histological analysis of mouse skeletal muscle

References:

**Project title: Regulation of skeletal muscle mass in health and disease**

**Laboratory:** Muscle biology and therapeutics

**Primary Supervisor(s):** Dr Paul Gregorevic and Dr Kevin Watt

**Contact:** paul.gregorevic@bakeridi.edu.au  phone: 8532-1224

**Research Focus:** Signalling pathways regulating skeletal muscle size, mechanisms promoting skeletal muscle atrophy in neuromuscular disease

**Keywords:** skeletal muscle, hypertrophy, atrophy,

**Project description:**
Physical frailty caused by loss of skeletal muscle mass and strength is one of the main factors contributing to disability, illness and premature death worldwide. The goal of our laboratory is to elucidate how the cellular mechanisms that regulate muscle development and adaptation become perturbed in muscle wasting, and to develop new therapeutic approaches to reverse loss of muscle mass, strength and metabolic function.

To do this we design and make recombinant viral vectors as a means to regulate and interrogate the cellular mechanisms controlling muscle adaptation in vivo with a combination of precision, efficacy, and speed not offered by other methods (Fig 1). Our expertise in using gene delivery technologies in skeletal muscle is unparalleled in Australia, and undertaking research with us is a unique opportunity to work with cutting edge methodology.

**Fig1. Schematic of rAAV production and application in vivo**

**Fig2. Schematic representation of the human Hippo signalling pathway (Harvey et al 3)**

This project will interrogate the role of the Hippo signalling pathway (Fig 2) as a novel regulator of skeletal muscle size in health and disease. This pathway is currently generating much study with numerous examples of publications in the very highest impact journals. The Hippo signalling pathway is also a key regulator of skeletal muscle size. However, the mechanisms that drive this remain unclear. Using gene delivery technologies and a number of disease relevant models e.g. cancer cachexia and amyotrophic lateral sclerosis we will assess the interactions between the Hippo signalling network, and other key signalling pathways, as mechanisms governing skeletal muscle size in health and disease. Research projects are available for Honours, Masters and PhD students.
Project related methods/skills/technologies:

- Range of molecular biology methods (Western blotting, q-RT-PCR, Histology, Cell culture, In vivo manipulation of gene expression)
- Design, manufacture and administration in vitro and in vivo of recombinant viral vectors.
- Animal models of disease. Small animal handling, and surgical procedures.

References:

**Project title:** Using gene therapy tools to study and treat skeletal muscle disease

**Laboratory:** Muscle Research and Therapeutics

**Primary Supervisor(s):** Dr Paul Gregorevic

**Contact:** paul.gregorevic@bakeridi.edu.au phone: 8532 - 1224

**Research Focus:** Developing molecular interventions to understand mechanisms controlling skeletal muscle attributes and treat conditions of muscle wasting.

**Keywords:** Skeletal muscle, cell signaling, growth regulation, gene therapy, wasting, cancer cachexia, sarcopenia, muscular dystrophy, aging.

**Project description:**

Physical frailty caused by loss of skeletal muscle mass and strength is one of the main factors contributing to disability, illness and premature death worldwide. Our goal is to elucidate how the cellular mechanisms that regulate muscle development and adaptation become perturbed in muscle wasting, and to develop new therapeutic approaches to reverse loss of muscle mass, strength and metabolic function.

What sets us apart is that we design and make recombinant viral vectors “in-house”, to regulate and interrogate the cellular mechanisms controlling muscle adaptation *in vivo* with a combination of precision, efficacy, and speed not offered by other methods. Our expertise in using gene delivery technologies to manipulate muscle is unparalleled in Australia, and undertaking research with us provides a unique opportunity to work with these cutting edge methods.

Opportunities are available to conduct studies within several of our research themes:

- How does the Transforming Growth Factor-β signalling network regulate muscle
- Novel genes that control skeletal muscle growth and wasting
- Using vector-based delivery of genome editing technology to study and treat disease
- Novel gene therapies for neuromuscular disorders, and wasting in chronic illness
- Using gene therapies to treat diabetes & diabetic complications.

Students are also welcome to discuss other projects that may fall outside of these themes, or involve building collaborations with other teams possessing complementary expertise. Research projects are available for Honours, Masters and PhD students.

![Recombinant viral vectors can be used as gene delivery tools to study the regulation of muscle attributes, and potentially to treat conditions associated with skeletal and cardiac muscle disease.](image)

**Figure 1:** Recombinant viral vectors can be used as gene delivery tools to study the regulation of muscle attributes, and potentially to treat conditions associated with skeletal and cardiac muscle disease.
**Project title:** Developing gene editing; tools for basic biology and the genesis of new therapeutics

**Laboratory:**
Muscle Research and Therapeutics Development

**Primary Supervisor(s):**
Paul Gregorevic, Timothy Colgan

**Contact:**
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**Research Focus:**
Designing and evaluating potential gene editing therapies for treating muscle wasting diseases and/or dissecting signaling pathways and gene expression control in muscle

**Keywords**
gene therapy, gene editing, mouse models, dystrophy, AAV, muscle, muscle wasting, CRISPR

**Project description:**
200-250 words (or less), synopsis of the project, highlighting the main features of the project (including the potential research question, aim, rationale). Images or graphics maybe added.

In the past three years the field of gene therapy has witnessed the emergence of a new type of technology, gene editing, which will reshape the interrogation of basic biology and approaches to medicine. Gene editing tools have been developed that enable double-stranded breaks to be made in genomic DNA. Through homologous or non-homologous repair, these breaks are resolved and the genomic DNA can be altered. Alternatively, these same tools have been modified in such a way to prevent them from cutting, yet allow them to retain their highly specific DNA sequence targeting abilities. These modified gene editing tools can now be used as artificial transcription factors, enabling regulatory control of a gene of interest. Never before has biology been better positioned to manipulate targeted endogenous genomic regions in a physiologically relevant context.

We, in collaboration with world-experts of this developing technology, are working to interrogate both in vitro and in vivo signaling pathways that are dysregulated in muscle wasting diseases and to use these tools to correct mutations that arise in inherited pathologies. By dissecting endogenous signaling or correcting mutations in these diseases, gene based interventions (which this lab specializes in) can be developed to address an unlimited spectrum of disorders.

The project will focus on design of genome editing constructs and packaging into recombinant viral vectors for subsequent testing in cell culture and mouse models of muscle disease. Projects are available at the Honours, MSc and PhD level.

**Project related methods/skills/technologies:**
- Use of gene-based therapies
- Working in a highly emergent field on cutting edge technology
- Use of mouse models
- General lab techniques (qPCR, Western blotting, cloning, DNA electrophoresis etc)

**References:**

**Project title:** The contribution of excess weight to socioeconomic inequalities in incident morbidity and mortality outcomes

**Laboratory:** Obesity and Population Health

**Primary Supervisor (s)** Dr Kathryn Backholer and A/Prof Anna Peeters

**Contact:** Kathryn.backholer@bakeridi.edu.au 85321276

**Research Focus:**
The Obesity and Population Health Unit’s research program aims to build the evidence base for public health policy regarding the prevention of obesity, diabetes and its consequent diseases. The approach of this Unit is to identify and fill evidence gaps in three key areas: (i) Social patterning of obesity and its related diseases, (ii) Health risks associated with obesity and diabetes including disability, and (iii) Health benefits associated with obesity and diabetes-related interventions. These three research strands are then combined using chronic disease modeling to interpret the associated population health implications.

**Keywords**
Socioeconomic inequalities, obesity, non-communicable disease, epidemiology

**Project description:**
The prevalence of obesity follows a socioeconomic pattern, such that those from more disadvantaged backgrounds are disproportionately represented. Obesity is a major risk factor for premature death and a range of health outcomes, including cardiovascular disease and diabetes, all of which are also socioeconomically distributed. However, the extent to which inequalities in excess weight (as indicated by body mass index or waist circumference) contribute to inequalities in death and ill health is unknown. An understanding of this will inform policy decisions for the prevention of obesity and its associated socioeconomic patterning.

The aim of this study will be to quantify the contribution of socioeconomic disparities in excess weight on inequalities in obesity related outcomes. The project will involve analyzing data from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), the largest Australian longitudinal population-based study examining the natural history of diabetes, pre-diabetes, heart disease and kidney disease.

**Project related methods/skills/technologies:**
- Regression and mediation analyses using statistical software, STATA
- General epidemiology and biostatistics skills

**References:**
**Project title:** Socioeconomic patterning of sugar sweetened beverage consumption over time

**Laboratory:** Obesity and Population Health

**Primary Supervisor (s)** Dr Kathryn Backholer and A/Prof Anna Peeters

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**Research Focus:**
The Obesity and Population Health Unit’s research program aims to build the evidence base for public health policy regarding the prevention of obesity, diabetes and its consequent diseases. The approach of this Unit is to identify and fill evidence gaps in three key areas: (i) Social patterning of obesity and its related diseases, (ii) Health risks associated with obesity and diabetes including disability, and (iii) Health benefits associated with obesity and diabetes-related interventions. These three research strands are then combined using chronic disease modeling to interpret the associated population health implications.

**Keywords**
Socioeconomic inequalities, obesity, non-communicable disease, epidemiology

**Project description:**
Compelling evidence demonstrates that regular consumption of sugar sweetened beverages (SSBs) is associated with adverse weight gain and a number of comorbid conditions, including diabetes and cardiovascular disease. SSBs are consumed in greater quantities by lower socioeconomic groups and are likely to contribute to socioeconomic inequalities in weight and disease. In Australia, several public health strategies have been implemented to reduce SSB consumption, however whether such initiatives have reduced population consumption of SSBs, and done so in an equitable manner, is unclear.

This study aims to examine trends in SSB consumption for children over time according to socioeconomic position. It will use nationally representative data from the Australian Health Survey’s and will involve the use of a number of different statistical methodologies. Findings from this study will be important to ensure that policies to reduce population consumption of SSBs are equitable in their effect across socioeconomic groups.

**Project related methods/skills/technologies:**
- Descriptive and regression methods using statistical software package, STATA
- General epidemiology and biostatistics skills
- Understanding of the policy environment for the prevention of population weight

**References:**
**Project title:** Social inequalities in trans fatty acid intake and the relationship with coronary heart disease

**Laboratory:** Obesity and Population Health

**Primary Supervisor(s):** Dr Kathryn Backholer and A/Prof Anna Peeters

**Contact:** Kathryn.backholer@bakeridi.edu.au 85321276

**Research Focus:**
The Obesity and Population Health Unit’s research program aims to build the evidence base for public health policy regarding the prevention of obesity, diabetes and its consequent diseases. The approach of this Unit is to identify and fill evidence gaps in three key areas: (i) Social patterning of obesity and its related diseases, (ii) Health risks associated with obesity and diabetes including disability, and (iii) Health benefits associated with obesity and diabetes-related interventions. These three research strands are then combined using chronic disease modeling to interpret the associated population health implications.

**Keywords**
Socioeconomic inequalities, non-communicable disease, epidemiology, cardiovascular disease, nutrition

**Project description:**
Trans fatty acids (TFAs) are unsaturated fatty acids that are produced in manufactured foods. Industrially produced TFA increases the risk of coronary heart disease, with over 1000 estimated deaths related to TFA consumption each year in Australia. In contrast, there are no known health benefits of industrially produced TFAs. Although the average intake of TFA is relatively low in Australia, it is likely that those from more disadvantaged backgrounds consume greater quantities. This unequal consumption may in turn contribute to the observed social patterning of coronary heart disease. Nevertheless, little is known regarding the socioeconomic patterning of TFA intake.

This project aims to examine the social distribution of TFA intake in Australia using a number of different socioeconomic position indicators. We will also aim to estimate the number of coronary heart disease deaths attributed to TFA intake in Australia by socioeconomic position.

This project will involve analysing data from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), the largest Australian longitudinal population-based study examining the natural history of diabetes, pre-diabetes, heart disease and kidney disease. The baseline AusDiab study involving physical testing and questionnaires was conducted in 1999-2000, with the second wave completed in 2005 and a third wave completed in 2012.

**Project related methods/skills/technologies:**
- Descriptive and regression statistical methodologies using statistical software, STATA
- General epidemiology and biostatistics skills
- Understanding of socioeconomic inequalities in health

**References:**
Project title: Health risks associated with a combined excess body mass index and waist circumference.

Laboratory: Obesity and Population Health

Primary Supervisor(s) Dr Stephanie Tanamas and A/Prof Anna Peeters

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Research Focus:
The Obesity and Population Health Unit’s research program aims to build the evidence base for public health policy regarding the prevention of obesity, diabetes and its consequent diseases. The approach of this Unit is to identify and fill evidence gaps in three key areas: (i) Social patterning of obesity and its related diseases, (ii) Health risks associated with obesity and diabetes including disability, and (iii) Health benefits associated with obesity and diabetes-related interventions. These three research strands are then combined using chronic disease modeling to interpret the associated population health implications.

Keywords
Waist circumference, obesity, body mass index, epidemiology

Project description:
The increasing health risks associated with obesity are well known. However, recent evidence suggests that using BMI alone may be insufficient to capture health risks associated with excess adiposity. The extent to which a combination of excess body mass index and waist circumference is associated with health risks over and above one anthropometric marker alone is unknown.

We aim to analyse the risk of incident diabetes, cardiovascular disease and mortality associated with combinations of excess body mass index and waist circumference.

Project related methods/skills/technologies:
The student will need an understanding of quantitative research methods and necessary statistical analysis skills. We will provide further training in epidemiology and biostatistics.

References:
**Project title:** Factors associated with a discordance in changes in weight and waist circumference

**Laboratory:** Obesity and Population Health

**Primary Supervisor (s)** Dr Stephanie Tanamas and A/Prof Anna Peeters

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**Research Focus:**
The Obesity and Population Health Unit’s research program aims to build the evidence base for public health policy regarding the prevention of obesity, diabetes and its consequent diseases. The approach of this Unit is to identify and fill evidence gaps in three key areas: (i) Social patterning of obesity and its related diseases, (ii) Health risks associated with obesity and diabetes including disability, and (iii) Health benefits associated with obesity and diabetes-related interventions. These three research strands are then combined using chronic disease modeling to interpret the associated population health implications.

**Keywords**
Waist circumference change, obesity, weight change, epidemiology

**Project description:**
We have preliminary data suggesting that weight gain and increasing waist circumference do not always occur together. We have also demonstrated previously that waist circumference appears to be increasing at a faster rate than weight. As excess waist circumference appears to have greater metabolic consequences than high body mass index it is important to understand factors that preferentially predict increasing waist circumference.

Here we aim to identify sub-populations in which changes in weight and waist circumference are not correlated. We additionally aim to identify preferential predictors of increasing waist circumference.

**Project related methods/skills/technologies:**
The student will need an understanding of quantitative research methods and necessary statistical analysis skills. We will provide further training in epidemiology and biostatistics.

**References:**