Session 1: Molecular Basis of Cardiovascular Disease

(1) Andrew Brown

Control of Cholesterol Synthesis

Andrew J. Brown

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If cholesterol in our bodies is not balanced, it is detrimental to human health. However, how cholesterol synthesis is controlled beyond HMGCR, the target of the statins, is poorly understood. We have found that other enzymes in the cholesterol synthesis pathway also control flux. 7-dehydrocholesterol reductase (DHCR7) is the terminal enzyme of cholesterol synthesis in the Kandutsch-Russell pathway, converting 7-dehydrocholesterol (7DHC) to cholesterol. In the absence of functional DHCR7, accumulation of 7DHC and a lack of cholesterol production leads to the developmental disorder, Smith-Lemli-Opitz syndrome (SLOS). We found that DHCR7 is subject to proteasomal degradation in the presence of cholesterol, resulting in decreased protein levels and activity in an example of end-product inhibition. The loss of enzymatic activity results in the accumulation of the substrate 7DHC, which leads to an increased production of vitamin D in skin cells. This study also identifies that statin treatment can ameliorate the low DHCR7 expression seen with common SLOS mutations in cultured cells. These findings highlight DHCR7 as an important regulatory switch between the production of these two vital molecules, cholesterol and vitamin D.

(2) Sally McCormick

Ribose-cysteine increases glutathione-based antioxidant status and reduces atherosclerosis in apoE-deficient mice

Sally McCormick

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Low levels of glutathione peroxidase (GPx) activity and elevated levels of oxidised phospholipids (OxPL) are associated with an increased risk of developing cardiovascular disease (CVD) (1,2). GPx functions to reduce OxPL to lipid alcohols and requires the cellular antioxidant, glutathione (GSH) as a cofactor. Ribose-cysteine is a cysteine analogue designed to increase the synthesis of GSH (3). We investigated the effect of ribose-cysteine on GSH, GPx, lipid peroxides, plasma lipids and atherosclerosis development in apolipoprotein E-deficient mice (apoE-/- mice). Female apoE-/- mice (12 weeks of age) were treated with 4 mg/day ribose-cysteine in drinking water for 8 weeks (n=9) or left untreated (n= 9). Blood, livers and aortae were harvested and GSH, GPx activity, F2-isoprostanes and plasma lipid concentrations were measured. Atherosclerotic lesion area in the aortic sinus and

brachiocephalic arch of treated and untreated mice were quantified. Ribose-cysteine treatment significantly increased GSH concentrations in the liver ($p<0.05$) and significantly increased GPx activity in the liver and erythrocytes of apoE-/-mice ($p<0.005$). F2-isoprostane levels were significantly reduced in the livers and arteries of apoE-/- mice ($p<0.05$ and $p<0.005$ respectively). Ribose-cysteine treatment was also associated with a significant decrease in total and low density lipoprotein (LDL) cholesterol ($p<0.05$) with no effect on other plasma lipids. Ribose-cysteine treatment significantly reduced atheroslcerosis lesion area in both the aortic sinus and brachiocephalic branch ($p<0.05$) of treated animals. Ribose-cysteine exerts an antioxidant effect by increasing GSH-based antioxidant status and lowering oxidised lipids. This combined with its LDL-lowering property, suggest that it might be an ideal supplementary intervention to increase protection against CVD.

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(3) Martin Fronius

Epithelial Na^+ channel and the endothelial glycocalyx mediate shear stress responsiveness in arteries

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The endothelial glycocalyx (EG) is important for vascular shear stress sensation and vascular responsiveness. The mechanisms and targets for the transduction of shear stress into cellular signals are poorly understood. The epithelial Na^+ channel (ENaC) is an emerging candidate, since its activity is regulated by shear stress and there is growing evidence of ENaC expression in arteries. The aim of our work is to reveal if and how ENaC and the EG interact to mediate vascular responsiveness.

Pressure myography experiments were performed to assess shear stress responses of isolated murine arteries and electrophysiological experiments of expressed ENaC were performed to gain insights into the mechanism involved in shear stress sensation. Hyaluronidase was used to degrade hyaluronic acid (HA, an essential component of extracellular matrices) and ENaC's contribution was addressed by application of amiloride.

Mouse carotid arteries dilated in response to increased intraluminal flow/shear stress. This response was augmented by amiloride confirming ENaC's role as vasoconstrictor. Hyaluronidase mimicked the amiloride response and the subsequent amiloride application was ineffective, indicating an interdependent activity of ENaC and the EG in arteries. This observation was confirmed in *Xenopus* oocytes – hyaluronidase treatment also decreased the shear-dependent ENaC activation. Site-directed mutagenesis revealed that *N*-glycosylated asparagines of ENaC are important for SF activation, suggesting that the attached glycans provide a connection to the extracellular matrix.

These experiments confirm an interdependent activity of ENaC and the EG that mediates shear stress sensation and vascular responsiveness. The interdependent activity relies on *N*-glycosylated asparagines of ENaC. Future studies may reveal whether impaired vascular responsiveness is caused by changes of the interdependent ENaC/EG activity.

(4) Monika Sharma

Recycling of apolipoprotein(a) after PlgRKT-mediated endocytosis of lipoprotein(a)

Monika Sharma[1], Greg Redpath[1], Michael J A Williams[2], Sally P A McCormick[1]
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Lipoprotein(a) (Lp(a)) is a low density lipoprotein (LDL)-like lipoprotein associated with an apolipoprotein(a) (apo(a)). The catabolic fate of Lp(a) in the liver remains controversial. Our study aims to determine the intracellular trafficking and catabolism of Lp(a) within HepG2 cells. Lp(a) was purified from normolipidemic healthy individuals using density gradient ultracentrifugation and fast protein liquid chromatography. HepG2 cells were treated with 5 $\mu g.mL^{-1}$ of Lp(a) and the uptake and intracellular localisation was studied using western blot analysis and confocal microscopy. Lp(a) was maximally internalised by HepG2 cells after 2 hours and mostly absent at 24 hours. Upon internalisation Lp(a), as detected by anti-apo(a) antibody, localises to early endosomes and the trans-Golgi network. Unlike LDL, Lp(a) did not colocalise with lysosomes. Golgi localisation of Lp(a) lead us to investigate the possibility of Lp(a) recycling. Interestingly, apo(a) component from the internalised Lp(a) was resecreted back into the cellular media which subsequently reformed Lp(a). Lp(a) uptake was not mediated by either the LDL receptor (LDLR) or the asialoglycoprotein receptor (ASGPR). Lp(a) was colocalised with the plasminogen receptor (KT) (PlgRKT) and Lp(a) internalisation was heavily reduced in haploid human fibroblast-like cells (HAP1) containing a PlgRKT knockout. These findings may have implications for the manipulation of Lp(a) levels.

(5) Hannah Prebble

Case study of the activation of macrophages in atherosclerotic plaque

Prebble, H. M.[1], Cross, S.[1], Aamir, R.[2], Anderson, N.G.[2], White, V.[3], Roake, J.A.[3], Healy, J.[1], Gieseg, S.P.[1,2]

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Atherosclerosis is a widespread disease which at present is identified when patients become symptomatic. Improved non-invasive imaging is required to gain an understanding of the morphological features that contribute to plaque vulnerability along with biochemical analysis to further understand how components such as macrophages are contributing to the disease progression. In this case study we used both imaging and biochemical analysis to study an atherosclerotic plaque removed by endarterectomy from a 65 year old male ex-smoker who present with transient ischemic attacks (TIAs). Our biochemical analysis centred around 7,8-dihydroneopterin, a biomarker or immune cell activation and inflammation in atherosclerotic plaque[1]. We also measured neopterin, the oxidative product of 7,8-dihydroneopterin, which is known to be elevated in patients with cardiovascular disease.

Stimulating the live plaque with PMA, a T cell mitogen, we tested whether it was possible to induce the production of 7,8-dihydroneopterin in resident macrophages. We kept the plaque alive in cell culture media supplemented with human serum for 4 days. Analysis of the lactate production each day indicated the ongoing cell viability. HPLC to measure 7,8-dihydroneopterin and neopterin showed increased levels of both compounds after stimulation, but the concentration produced varied across plaque sections. These data show the macrophages were activated and that there is an oxidative environment within the plaque. X-ray imaging of the plaque using a MARS Spectral CT (a novel technique which produces a quantitative material decomposition of the plaque) showed that some sections of the plaque were highly calcified. The sections producing the lowest concentration of 7,8-dihydroneopterin contained the greatest volume of calcium. This suggests there may be a lower abundance of macrophages in the highly calcified tissue.

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Session 2: Genetics and Biomarkers

(1) Annika Winbo

(2) Vicky Cameron

Heart Disease Biomarkers: An Update

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Mortality rates from heart disease peaked in the late 1960s and have since fallen markedly (1), through a combination of reduced smoking rates, better management of cardiovascular risk and advances in treatment. Among life-saving advances in cardiology over the past 15-20 years, biomarker development by the Christchurch Heart Institute has played a key role in three. These include: chest pain pathways for early discharge of low risk chest pain; use of brain natriuretic peptide (BNP) levels for diagnosis of heart failure; and BNP use to guide treatment of heart failure. The latter three of these advances, all involve biomarkers that were developed in Christchurch. However, cardiovascular diseases still account for 37% of mortality in New Zealand / Aotearoa (2), and there is a need for better predictors to identify individuals in the population at impending risk of an acute cardiovascular event, to prioritise clinical monitoring and risk factor management to those people most at risk. Our understanding of inherited risk of cardiovascular diseases has accelerated since the release of the human genome sequence in 2000, leading to a new appreciation of non-coding DNA in disease susceptibility (3). This is fuelling the discovery of potential biomarkers in circulating non-coding RNAs (transcripts from non-coding DNA), such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs).

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(3) Rajesh Katare

Circulating cardiovascular microRNAs as diagnostic biomarkers for early diagnosis of diabetic heart disease

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Heart disease in diabetics develops at a much early stage, but is often unrecognized due to the absence of pathognomonic signs, thereby restricting its early detection and active therapeutic management. Identifying the early modulators in diabetic heart disease (DHD) will not only help in early detection of the disease, but also allow sufficient time for optimization of the treatment. Recently microRNAs (miRs) are gaining interest as diagnostic biomarkers for several diseases including cardiovascular diseases due to their stability in circulation and tissue specificity. However, to date, their diagnostic potential in DHD has not been studied. In this seminar, I will presenting our new results from pre-clinical and clinical trials showing dysregulation of cardiac enriched miRs in diabetic heart. Importantly, dysregulation of miRs occurred well before the development of morphological or structural changes in the diabetic heart. These findings provide first evidence that miRs may be the early modulators of DHD and that measuring the level of circulating miRs could become a valuable diagnostic tool for early detection of DHD.

(4) Steve Gieseg

Macrophage markers and plaque imaging

Gieseg S.P.[1,2], Aamira, A.[2,4], Anderson, N.[2,4], Buttlet, A.[2,5], Chen, A.[1], Cross, S.P.[1], Healy, J.[1,4], Katouah, H.[1], Marks, E.C.A.[1], Palmer, B.[6], Prebble, H.M[1], Roake, J.A.[3], White, V.[3]

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Cardiovascular disease (CVD) is extremely difficult to diagnosis until a patient has reached a significant level of artery occlusion or atheroma instability. Measurement of subclinical vascular disease to identify at risk patients relies of indirect markers such as LDL-cholesterol. There is a lack of specific markers of atheroma growth or stability. Similarly, the measurement of treatment efficacy relies either on extreme end points, such as death or indirect markers e.g. LDL-c, blood pressure. Current non-invasive imaging modalities lack either the resolution or molecular discernment to detect sub-clinical atherosclerotic plaques to further quantify plaque pathology within a patient.

Macrophages appear to be the primary antagonist in the development of atherosclerotic plaques. Macrophage mediated low density lipoprotein oxidation and uptake generates the cholesterol rich foam cells which dominate the lipid core of advanced atherosclerotic plaques. Immune activation of macrophages by interferon releases the pterin 7,8-dihydroneopterin which is oxidised to either neopterin or xanthopterin depending on the oxidants present[1]. The presence of neopterin and 7,8-dihydroneopterin are strongly associated with CVD. We here show that neopterin is a primary product of 7,8-dihydroneopterin oxidation suggesting that the measurement of neopterin and 7,8-dihydroneopterin in CVD patients may provide a measure of plaque inflammatory status and superoxide generation. Combining the pterin measurement with the levels of plasma sFLt-1 further increases the correlation with CVD patients. sFlt-1 binds VEGF, a promotor of angiogenesis. Plasma sFlt-1 correlated with plasma 7,8-dihydroneopterin but not neopterin indicating a link between macrophage activation and ischemia, a driver of plasma sFlt-1 levels.

Further confirmation of these process will require the development of histology levels imaging of plaque with patients. Imaging of excised plaques before cell culturing using a MARS-spectral-X-ray-CT machine[2] has allowed the positive identification of lipid cores within excised carotid plaques before cell culturing of segments at high resolution. The immune cell activity has shown some correlation with the plaque morphology as imaged by the MARS-spectral-CT.

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Session 3: Environmental Influences on Heart Disease

(1) Kirsten Coppell

Can primary care contribute to halting and reversing the diabetes epidemic?

Kirsten Coppell

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Dysglycaemia is a well-recognised risk factor for cardiovascular disease, and cardiovascular and all-cause mortality. In New Zealand the prevalence of diabetes is 7% and the prevalence of prediabetes is 26% (1), and cardiovascular diseases including diabetes accounts for 17% of health loss (2). There is an urgent need to implement effective prediabetes management strategies to reduce the increasing health and economic costs of New Zealand's growing diabetes epidemic. Although good clinical trial data suggest that implementation of intensive lifestyle advice has the potential to approximately halve the risk of progression from prediabetes to type 2 diabetes, (3) similar results have not been readily achieved in 'real world' settings. In New Zealand, screening for prediabetes is a component of cardiovascular risk assessment, yet in most regions, widespread effective lifestyle advice is not available for those identified as having prediabetes. Part of the reason is that nutritional knowledge among primary care providers is often limited, yet they are expected to deliver effective lifestyle advice. Practice nurses are ideally suited to lead lifestyle changes to prevent progression from prediabetes to diabetes. A structured practice nurse-delivered nutrition intervention programme based on a brief dietary assessment may be a pragmatic, more inclusive alternative to the more intensive and more expensive programmes provided in the renown diabetes lifestyle prevention trials.

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(2) Anna Rolleston

Using a Maori philosophy to formulate meaningful questions to address heart disease: from the laboratory to the community

Anna Rolleston

The Cardiac Clinic, The University of Auckland

The purpose of this presentation is to understand how a Maori worldview can be interfaced with traditional research to produce meaningful questions and processes that benefit communities. The

disparate worlds of Maori and medical/clinical, can be acknowledged in practice and research without loosing the foundations upon which each is built. A practical step-by-step method will be presented that will illustrate how the interfaced approach can be easily utilised across a range of scientific investigations using examples from current research.

(3) Sandra Mandic

Built Environment, Active Transport and Cardiovascular Disease Prevention

Mandic, S.[1]

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Physical inactivity and sedentary lifestyles are global public health problems. Recently, ecological models have been developed to take into account individual, socio-cultural, environmental and policy influences on physical activity. Built environment characteristics including open spaces/parks, urban design/land use, transportation infrastructure, location and design of schools and workplaces and home design influence all four domains of physical activity (recreation, transportation, occupation and domestic activities).

Built environment features such as walkable community design, presence of pedestrian and bicycle facilities and perceived accessibility and convenience promote active modes of transport such as walking and cycling. Active transport to different destinations is a convenient way to incorporate physical activity into everyday life. The shift from automobile transport to active transport results in substantial health benefits primarily from increased physical activity levels and clearly outweigh estimated negative effects of traffic accidents and air pollution exposure.(1)

Although some commonalities may exist across high income countries, how the environment impacts physical activity and sedentary behaviour is not universal. For example, perceptions and features of the built environment, climate/weather, social norms and policies can influence factors such as availability and use of public transport and private vehicle ownership. Therefore, understanding of the local context is essential for identifying effective intervention strategies to promote active transport. In Dunedin, Built Environment and Active Transport to School: BEATS Study examines adolescents' active transport to school using an ecological model (www.otago.ac.nz/beats).(2) The findings are providing timely and relevant information for schools, city councils, transport agencies and land planners and will help inform future changes to the built environment and policy development.

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Session 4: The XX Factor in Heart Disease

(1) Alison Heather

Ischemic Heart Disease in Women

Heather, Alison K.

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Ischemic heart disease, also known as coronary artery disease, continues to be the leading cause of death among women in New Zealand, as well as other Western countries including Australia and the United States. Ischemic heart disease accounts for ~ 1 of every 3 deaths of New Zealand women. Cardiovascular risk factors unique to women have only recently been recognized, and moreover, traditional risk factors have recently been shown to have greater impact on women. Consequently, women suffer more disability and poorer clinical outcomes. Focused diagnostic and therapeutic strategies unique to women are thus needed. Hormone replacement therapy is an option but, to date, there remains no evidence that hormone replacement therapy confers any cardiovascular protection (or harm). Importantly, when considering initiation of hormone replacement, it is important to consider a women's age, number of years since menopause, and a number of cardiovascular risk factors. There is growing consensus that the benefit to risk profile for hormone therapy is high for healthy, low-risk women initiating treatment within 15 years of menopause or under age 60. However, special consideration is needed for women outside these boundaries or for those that have risk factors for cardiovascular disease. In my laboratory, we have increasing evidence that estradiol therapy, initiated well after atherosclerotic lesions have formed, accelerates calcification and thus could help explain some of the adverse clinical findings reported for hormone therapy in older women.

(2) Lea Delbridge

Myocardial vulnerability – estrogens, androgens & arrhythmogenesis

Delbridge, L.M.D

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For both women and men heart disease is the major cause of death – yet the disease onset and endpoint characteristics differ between the sexes. Observational and epidemiological studies support the concept of a cardioprotective role for estrogen. However findings from randomized controlled trials have not demonstrated estrogen supplement cardiac benefit – and actually identified increased risk for some women. A more complete understanding of the actions of estrogens and androgens at the myocardial level is required to inform future therapeutic interventions of sex-selective value. Experimentally, we have demonstrated that at the most fundamental level, the processes of electromechanical transduction in female and male

cardiomyocytes differ. These differences are linked with contrasting arrhythmogenic vulnerability states and responsiveness to inotropic intervention.

Most recently, we have established that the myocardium and pericardial adipose depots are aromatase-expressing tissues. Thus, there is important capacity for the local cardiac conversion of androgens to estrogens. In obesity, when cardiac adiposity is markedly increased, the impact of aromatase-mediated shifts in androgen-estrogen balance may be significant. There is accumulating evidence to support the case for operation of a local cardiac androgen-estrogen system of pathophysiologic consequence. This local sex steroid axis may offer crucial clinical interventional leverage tailored differently for women and men.

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(3) Jinny Willis

Heart Disease Risk in Women with Diabetes: Double Jeopardy?

Willis, J., Scott, R.S.

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Diabetes mellitus is a disorder of absolute or relative insulin deficiency associated with significant microvascular and macrovascular complications. The Ministry of Health Virtual Diabetes Register (VDR) includes all individuals identified by a number of diabetes flags including diabetes-associated hospital admissions, prescriptions for diabetes-related therapies, attendance at specialist or management clinics, laboratory tests for HbA1c and retinal screening. As at December 2015, the VDR enumerated around 260 000 New Zealanders with diabetes. Diabetes is associated with increased prevalence of established risk factors for cardiovascular disease (CVD) such as overweight or obesity, hypertension, and dyslipidaemia. It has been reported that women with diabetes have greater relative risk of CVD than men with diabetes compared with individuals without diabetes.

The Canterbury Diabetes Register was established via a community survey based in retail pharmacies and included all individuals using insulin as a long-term modality at the prevalence date of 1 January 1984. At 20 year follow-up in 2004, 525 deaths had occurred among 966 cases. A total of 320 deaths were certified to CVD, accounting for the majority of excess mortality across all ages. The standardised mortality ratios (SMRs) for CVD were 3.73 (95% CI: 3.16-4.30) and 3.27 (95% CI: 2.76-3.78) respectively for women and men respectively. SMRs for CVD were greater in women than men across all age groups.

There is limited evidence to suggest that more attention needs to be paid to management of modifiable CVD risk factors in women with diabetes. In the context of increasing numbers of individuals with diabetes, early CVD risk assessment and initiation of therapy to modify risk factors in this group is a priority.

Session 5: Cardiac Function and Dysfunction

(1) David Eisner

Cardiac transverse tubules: from function to failure.

Eisner, D.A., Caldwell, J.L., Trafford, A.W. & Dibb, K.M.

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Contraction of cardiac muscle requires synchronized release of calcium from the sarcoplasmic reticulum. The diameter of mammalian ventricular myocytes is sufficiently large that diffusion of calcium from the periphery of the cell would be too slow, a problem which is overcome by the existence of transverse (t-) tubules. In this talk, I will consider two aspects of t-tubular function.

Until recently, it had been though that, unlike the ventricle, atrial myocytes had no t-tubules. We have found, however, that atrial cells from larger mammals have well-developed t-tubule networks. Interestingly, the diameter of atrial cells from larger mammals is greater than those from smaller ones and this may provide a need for t-tubules. In experiments on sheep atrial myocytes we find that heart failure (induced by rapid ventricular pacing) results in the disappearance of virtually all t-tubules. As a result, calcium is released initially at the periphery of the cell rather than uniformly.

The other area of research concerns the factors that are responsible for maintaining this regular network of t-tubules. One candidate protein is Amphiphysin II. We find that levels of Amphiphysin II fall accompanying the loss of t-tubules observed in heart failure, Specific knockdown of Amphiphysin II with SiRNA leads to t-tubule loss. I will discuss the role of Amphiphysin II and other proteins in the regulation of t-tubules.

(2) Rebecca Ritchie

Targeting physiological growth signalling as a novel therapy for diabetic cardiomyopathy

Ritchie, R.H.

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'Diabetic cardiomyopathy' is characterized by impaired left ventricular (LV) diastolic function, cardiomyocyte hypertrophy, LV fibrosis & cardiomyocyte apoptosis. Elevated oxidative stress and LV generation of the reactive oxygen species (ROS), superoxide, are considered likely contributors to this pathophysiology. Phosphoinositide 3-kinase (PI3K), downstream of the tyrosine kinase receptor insulin-like growth factor-1 receptor (IGF-1R), mediates membrane trafficking, cell growth and survival. Our laboratory has undertaken a number of studies aimed at assessing whether cardiac-selective approaches to enhance physiological growth signalling in the diabetic heart, or targeting LV superoxide via chronic supplementation of coenzyme Q_{10}, protect against diabetes-induced dysfunction and remodelling, using a range of preclinical *in vivo* models of both type 1 and type 2 diabetes. We have now demonstrated that diabetes-induced LV diastolic dysfunction,

cardiomyocyte hypertrophy and cardiac fibrosis, in addition to cardiac ROS levels, are attenuated by these approaches, revealing firstly that inappropriate LV superoxide levels thus appear to play a key causal role in triggering diabetic cardiomyopathy. Secondly, we have also revealed that cardioselective transgenic upregulation of IGF-1R/PI3K signalling prevents diabetic cardiomyopathy, with evidence of suppression of LV ROS levels. Amelioration of both diabetes-induced maladaptive cardiac glucose metabolism and oxidative stress accompanies preservation of LV structure and function in the diabetic heart. Finally, we have also demonstrated that enhanced cardiac IGF-1R/PI3K signalling not only prevents diabetic cardiomyopathy in mice, but is also effective when the treatment intervention is delayed intervention until after LV diastolic dysfunction is evident. Cardiac-selective PI3K adeno-associated viral (AAV) gene therapy administered after 8 weeks of untreated diabetes (when LV diastolic dysfunction is already evident), limits diabetic cardiomyopathy. Ultimately, strategies to enhance the cardioprotective effects of IGF-1R/PI3K signalling may represent attractive adjunct therapies for diabetic heart failure.

(3) Regis Lamberts

Changes in β-AR signalling in the human diabetic heart

Recently, we showed that right atrial muscles from type 2 diabetic patients with preserved ejection fraction and coronary artery disease had preserved contractile function with impaired relaxation. These change in the human atrial myocardium occurred despite an unexpectedly increase in the ratio of sarcoplasmic reticulum Ca^{2+} ATPase/phospholamban protein expression, which would promote an increased cytosolic Ca^{2+} removal and hence improved relaxation. In addition, we demonstrated that human right atrial cardiac muscles from type 2 diabetic patients with preserved ejection fraction are unresponsive to β-adrenergic stimulation despite no change in β_1-adrenoreceptor expression levels, suggesting impaired downstream β-adrenergic modulation. In this study we further determined the expression of downstream β-adrenergic and Ca^{2+} handling proteins in the right atrium and left ventricle of the human diabetic heart. Determination of human β-adrenergic signalling in the diabetic heart is clinically relevant as others have shown that traditional treatment of diabetic patients with β-adrenergic blockers is of benefit, however to a lesser extent than in non-diabetic patients.

(4) Luis Gonano

RyR2 dysfunction and Digitalis Toxicity.

Luis Alberto Gonano[1-2], Marisa Sepúlveda[1], Malena Morell[1], Tamara Tottef[1], Peter P Jones[2], Thomas G Back[3], S R Wayne Chen[4], Alicia Mattiazzi[1] and Martin Vila Petroff[1].

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Digitalis or Cardiotonic glycosides inhibit the sarcolemmal Na^+/K^+-ATPase and cause an increase in intracellular Na^+, which reduces Ca^{++} extrusion through the Na^+/Ca^{++} exchanger. The result is an increase in sarcoplasmic reticulum (SR) Ca^{++} load and cardiac contractility. However, these compounds have associated arrhythmic effects due to the occurrence of spontaneous Ca^{++} release from the sarcoplasmic reticulum to the cytosol. We explore the mechanisms implicated in digitalis-induced arrhythmias and a possible therapeutic approach to prevent it adverse effect.

Session 6: Free Abstract Session

(1) Max Pinkham

Weight gain in ovariectomized female rats does not exacerbate heart failure development following myocardial infarction

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In women, the incidence of heart failure (HF) dramatically increases post-menopause, following a significant reduction in circulating ovarian hormones. Therefore, ovarian hormones are thought to have direct cardioprotective effects. However, body weight (BW) and visceral adiposity also tend to increase in women post-menopause. Increased adiposity is associated with a greater incidence of HF and a worse prognosis. Therefore, we investigated whether an increase in BW and adiposity in ovariectomized female rats aggravates HF development following myocardial infarction (MI). Female rats were ovariectomised (OVX) and a subset were weight controlled (WC) by restricting food intake, thereby maintaining similar BW as age-matched ovary-intact female rats. Two weeks following OVX, MI or sham-MI surgery was performed and eight weeks later left ventricular (LV) function was assessed. Subsequently, heart, lung and adipose tissues were removed and weighed. MI sizes were similar across all three MI groups. BW and visceral fat weights were significantly increased in OVX groups compared to OVX-WC groups and ovary-intact groups. MI did not affect BW and visceral fat weights. Cardiac output was preserved in ovary-intact MI group (114 ± 11 $ml \cdot min^{-1} \cdot kg^{-1}$) compared to sham MI group (125 ± 9 $ml \cdot min^{-1} \cdot kg^{-1}$, $P > 0.05$). In comparison, cardiac output was decreased in OVX MI group (76 ± 7 $ml \cdot min^{-1} \cdot kg^{-1}$) and OVX-WC group (75 ± 9 $ml \cdot min^{-1} \cdot kg^{-1}$) compared to respective sham MI group and also ovary-intact MI group ($P < 0.05$). Load independent indicators of LV systolic function were preserved in ovary-intact MI group and significantly reduced in both OVX MI group and OVX-WC MI group. Indicators of LV diastolic function were not different between MI groups. The results suggest that weight gain post-ovariectomy does not significantly affect heart failure development following MI. However, the loss of circulating ovarian hormones in female rats is associated with worse LV systolic function post-MI.

(2) Joanne Harrison

Novel organic carbon monoxide releasing molecules protect the heart against ischaemia reperfusion injury.

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Cardiac and cardiovascular interventions are the most common major clinical procedures world-wide but often require a temporary cessation of blood flow which can result in ischaemia reperfusion injury to the heart. There is an opportunity and demand for advancements in pharmaceutical technology to improve cardiac viability, structure and function following ischaemia inducing surgical procedures. Low dose carbon monoxide (CO) gas has been shown to improve cell, tissue and organ function in numerous studies including human cardiovascular ischaemic surgery. However, concerns over the handling of this potentially toxic gas and technical difficulties surrounding its administration have limited its clinical application to date. We have developed and patented water soluble, organic CO releasing molecules (oCOms) as an easily controlled and safe method of administering CO. These oCOms avoid the use of toxic metal cores which have hindered the development of CO donor compounds by other groups.
Prophylactic use of oCOm-21 (3 & 10µM) was assessed in isolated Langendorff perfused rat hearts subjected to a subsequent 30 min warm (37°C) global zero flow ischaemia followed by reperfusion. Left ventricular (LV) mechanical indices, measured using an intraventricular balloon-pressure transducer, and coronary effluent flow were recorded at a set LV end-diastolic pressure of 10 mm Hg. LV function was characterised by LV developed pressure (LVDP) derived from the difference between peak systolic pressure and end diastolic pressure. The peak rates of rise and fall in the first derivative of LV pressure (dP/dtmax and dP/dtmin, respectively) and heart rate (HR) were measured using Chart v6. oCOm-21 (3 µM) administration 10 minutes prior to the ischaemic episode significantly attenuated the extent of ischaemic reduction in LVDP, dP/dt max and dP/dt min coronary flow rate compared to controls. There was no significant effect on heart rate. oCOm-21 shows significant potential to protect the heart from ischaemia reperfusion injury and warrants further investigation in other models of ischaemia reperfusion injury.

(3) Denis Loiselle

Can Dietary Supplementation with Omega-3 Fish-Oils *Really* Improve the Pumping Efficiency of the Heart?

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The pumping efficiency (ε) of the heart, or its tissues, is given by the First Law of Thermodynamics: $\varepsilon = W/(W + Q)$, where *W* is external (pressure-volume or force-length) work and *Q* is heat. Heat arises from inefficient capture of the Gibbs Free Energy of ATP hydrolysis (ΔG_{ATP}) – an obligatory consequence of the Second Law of Thermodynamics if work is to be performed. It comprises the wasted energy from each of the four major cellular sources: basal metabolism, mitochondrial oxidative phosphorylation, Ca^{2+}-triggered activation, and contraction-related cycling of the actin-myosin cross-bridges.
Over the past decade, there have been a number of publications claiming many-fold improvements of cardiac pumping efficiency as a consequence of a period of dietary supplementation with Omega-3 fish-oils. We have been sceptical of such claims and, in consequence, have explored the question:

"*Can the pumping efficiency of the heart be quadrupled, tripled, or even doubled by a fish-oil-supplemented diet or by any other intervention?*"
Our theoretical investigation has led us to answer the question in the negative.

(4) Rohit Ramchandra

Divergent changes in cardiac sympathetic nerve activity and norepinephrine spillover in heart failure

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One of the clinical features of patients with heart failure (HF) is increased levels of cardiac and renal norepinephrine (NE) spillover, both of which are strong independent predictors of mortality. The prevailing dogma is that this increase in NE spillover is predominantly driven by an increase in sympathetic nerve activity. We hypothesized that these increases in NE spillover in HF depend not only on increases in sympathetic nerve activity (SNA), but also on changes in the mechanisms controlling NE release and re-uptake. Such changes would lead to differences between the increases in directly recorded SNA and NE spillover to the heart and kidney in HF.
To test this hypothesis, experiments were conducted in conscious sheep implanted with cardiac SNA (CSNA) and renal SNA (RSNA) recording electrodes. Together with recordings of CSNA, RSNA and arterial pressure, the spillover of NE from both the heart and the kidney was determined. Compared with normal sheep, in animals with HF there was a two-fold increase in CSNA (42±8 vs. 95±11 bursts/100hb, $p<0.05$), but a five-fold increase in cardiac NE spillover (3.1±0.8 vs. 18.1±4.1 pmol/min, $p<0.05$). Baroreflex control of cardiac NE spillover was impaired in the HF animals in contrast to normal animals. In HF, RSNA was unchanged (95±8 bursts/100hb), but interestingly spillover of NE from the kidney was significantly increased (10.1±2.4 vs. 52.5±10.2 pmol/min, $p<0.05$). Interestingly, when directly recorded CSNA was completely inhibited using phenylephrine, there was a significant decrease in cardiac spillover of NE in the normal animals, but during HF, there was no change in cardiac spillover of NE. The mismatch between SNA and NE spillover in the heart and kidney in ovine HF suggests that other mechanisms, such as changes in activity of the NE transporter, may play an important role in mediating the changes in NE spillover.

(5) Pete Jones

FKBPs facilitate the termination of spontaneous Ca^{2+} release in wild type RyR2 but not CPVT mutant RyR2

ABSTRACT
FK506 binding proteins 12.6 (FKBP12.6) and 12 (FKBP12) tightly associate with the cardiac ryanodine receptor (RyR2). Studies suggest that dissociation of FKBP12.6 from mutant forms of RyR2 contributes to store overload induced Ca^{2+} release (SOICR), and Ca^{2+} triggered arrhythmias. However, these findings are controversial. Previous studies focused on the effect of FKBP12.6 on the initiation of SOICR and did not explore changes in the termination of Ca^{2+} release. Less is known about FKBP12. We aimed to determine the effect of FKBP12.6 and FKBP12 on the termination of SOICR. Using single cell imaging, in cells expressing RyR2 wild type, we found that FKBP12.6 and FKBP12 significantly increase the termination threshold of SOICR without changing the activation

threshold of SOICR. This effect, dependent on the association of each FKBP with RyR2, reduced the magnitude of Ca^{2+} release but had no effect on the propensity for SOICR. In contrast neither FKBP12.6 nor FKBP12 was able to regulate an arrhythmogenic variant of RyR2, despite a conserved protein interaction. Our results suggest that both FKBP12.6 and FKBP12 play critical roles in regulating RyR2 function by facilitating the termination of SOICR. The inability of FKBPs to mediate a similar effect on the mutant RyR2 represents a novel mechanism by which mutations within RyR2 lead to arrhythmia.

(6) Akash Deep Chakraborty

Regulation of RyR2 by Protein Kinase CK2

Akash Deep Chakraborty, Janet McLay, Peter Jones

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Under normal conditions, depolarization of cardiac myocytes activates the L-type Ca^{2+} channels, leading to a small Ca^{2+} influx which then activates RyR2, resulting in a large Ca^{2+} release from the sarcoplasmic reticulum (SR) and subsequent muscle contraction. This process is known as Ca^{2+}-induced Ca^{2+} release (CICR). Spontaneous SR Ca^{2+} release via RyR2 can occur under the conditions of SR Ca^{2+} overload, a process also termed store-overload–induced Ca^{2+} release (SOICR), an onset mechanism for arrhythmia. Phosphorylation of RyR2 by kinases (PKA, CaMKII, etc) have been shown to increase the propensity for SOICR. Calsequestrin (CSQ2), which is a Ca^{2+} buffering protein is an important regulator of RyR2, is closely linked to Protein Kinase CK2. CSQ2 has 3 phosphorylation sites known to be phosphorylated by the kinase. CK2 knockdown has been found to lower CSQ2 phosphorylation, altering the open probability (Po) of RyR2 and changing its function. However, a direct role for CK2 regulation of RyR2 has not yet been identified.
We have used Ca^{2+} imaging to show an increase in the activity of RyR2 (propensity of SOICR) when CK2 is inhibited. CK2 inhibition was achieved using an inhibitor (CX-4945) and CK2 specific siRNA. We have subsequently identified several new CK2 phosphorylation sites within RyR2 using mass spectrometry following *in-vitro* phosphorylation of RyR2. Mutants to specific sites have been generated to study the effects of phosphorylation and dephosphorylation of RyR2 by CK2. Our data indicate a novel role for CK2 in regulating the function of RyR2.

(7) Lorna Daniels

The effects of CaMKII inhibition on post-rest behavior and spontaneous contractions in isolated Zucker diabetic fatty rat cardiac muscle

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Calmodulin-dependent protein kinase II (CaMKII) is a multifunctional serine-threonine kinase reported to be activated in human patients and animal models of diabetes. Over-activation of CaMKII has been shown to result in disturbed calcium (Ca^{2+}) handling due to hyperphosphoyrlation of ryanodine receptor (RyR2) at Ser2814, causing Ca^{2+} leak from RyR2. Post-rest contractile behavior (PR) of isolated cardiac muscle indicates the capacity of the sarcoplasmic reticulum (SR) to store and release Ca^{2+}. Therefore we hypothesized that inhibition of CaMKII activity would improve PR in heart tissue from the Zucker diabetic fatty rat (ZDF), a model of type 2 diabetes. *Methods*- Cardiac muscles (trabeculae) from the right ventricle were isolated from 20 week old type 2 diabetic ZDF and control (CTRL) rats. Myocardial force measurements were performed in the presence of an inhibitor of CaMKII activity (KN93) or a peptide analogue with no CaMKII inhibitory effects (KN-92). PR was measured at a basal stimulation frequency of 2 Hz and contractile parameters of the first twitch after increasing rest intervals (10-120s) were evaluated. *Results*- Trabeculae isolated from the diabetic ZDF rats had reduced contractile force (F_{dev} and dF/dt_{max}) across all stimulation frequencies, alongside impaired relaxation (dF/dt_{min}). Inhibition of CaMKII with KN93 in the diabetic trabeculae significantly improved contractile force and relaxation kinetics. Despite the impaired contractility in the diabetic ZDF rat, there was no difference observed in the PR between the diabetic ZDF rats and CTRL. However, cardiac muscle from the diabetic ZDF rats, but not the CTRL rats exhibited spontaneous contractions indicating some form of Ca^{2+} mishandling in the diabetic heart. CaMKII inhibition with KN93 had no effect on reducing the occurrence of spontaneous contractions. *Conclusion*- The results indicate that CaMKII inhibition with KN93 is beneficial in preserving contraction and relaxation in isolated diabetic cardiac muscle. Our observations suggest a potential therapeutic role for CaMKII inhibitors in improving diabetic cardiac myocardial function. However there was no effect of KN93 on reducing the occurrence or severity of spontaneous contractions in the diabetic ZDF rat trabeculae. Therefore the use of other CaMKII inhibitors (eg. AIP) is warranted to further investigate the effects of CaMKII inhibition on Ca^{2+} handling in the type 2 diabetic heart.

Session 7: Heart Modelling- From *in vivo* to *in silico* and back

(1) Edmund Crampin

Calcium signalling in cardiac hypertrophy: from models to measurements and back again

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Calcium signals coordinate contraction of the heart with each heart beat. Calcium signals arising from hormonal stimulation also regulate growth of heart cells, for example during development and also in hypertrophic heart disease. Given that these different calcium signals appear to coincide in the cell, how do they not interfere with one another? I will discuss our recent attempts to address this issue by constructing data-driven mathematical models of calcium signalling, from the scale of individual ion channels up to cell-level calcium dynamics. This forms part of a broader initiative in cardiac cell systems biology to understand the interactions between cellular signaling, mechanical and metabolic pathways in the heart in health and disease.

(2) Renee Miller

A Method for Estimating Anisotropic Cardiac Stiffness Non-invasively

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Myocardial stiffness is an important determinant of cardiac function, and significant increases in global stiffness are thought to be associated with diastolic heart failure (1). However, stiffness can only be measured invasively using a catheter to measure pressure. Magnetic resonance elastography (MRE) is a non-invasive technique to estimate stiffness of soft tissues without the need for pressure recordings. MRE is a three stage process in which 1) external actuators are used to generate acoustic distortional waves in the tissue, 2) the wave displacements are quantified using phase-contrast MRI, and 3) the displacement information is converted into stiffness maps using mechanical modeling.

A method was developed which combines MRE images with anatomical cine and diffusion tensor images, which provide information about wave propagation, geometry and muscle fibre orientation, respectively. A finite element model was customised to epicardial and endocardial contours from the anatomical images. MRE wave displacements and fibre orientations were interpolated at registered coordinates in the model. Then, the mechanical equilibrium equations were solved to simulate the harmonic motion. The stiffness parameters describing the material were iteratively changed until the difference between the simulated displacements and MRE displacements was minimised.

The method was successfully tested with phantom data as well as simulated anisotropic data. The estimated stiffness parameter (Young's modulus = 16.76 kPa) was very close to the actual stiffness (16.35 kPa) of the phantom. The method was then employed with pig in-vivo cardiac data which was collected before and after inducing diastolic heart failure through a renal wrapping procedure. However, the method should be optimised further for use with in-vivo animal data, which provides significant hurdles such as poor image resolution and bulk cardiac motion. In the future, this method could help our understanding of the changes in anisotropic stiffness that occur as a result of diastolic heart failure.

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(3) Kim Mellor

Bioenergetics of the diseased heart

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Cardiac dysfunction in disease states is often linked to cardiomyocyte metabolic and mechanical alterations. The role of mechano-energetic disturbances in primary cardiac pathology is not well understood, particularly in the context of cardiac hypertrophy. Increased heart size is a major independent risk factor for heart failure and mortality and abnormal heart growth is linked with structural disorganisation of subcellular components and adverse metabolic adaptations. Our studies utilise a rat model of primary cardiac hypertrophy, the hypertrophic heart rat (HHR), to assess the mechano-energetic performance of cardiac trabeculae. Cardiac mechanical efficiency (ε = work/(work + heat)) is a primary determinant of mechano-energetic status, measured using simultaneous acquisition of work output and heat production data using a work loop calorimeter. Normotensive cardiac hypertrophy is associated with increased mechanical efficiency but reduced muscle contractile performance. Dramatic suppression of activation heat in trabeculae from hypertrophic hearts suggests that alterations in cellular heat sources such as SERCA2a may play an important role in determining mechano-energetic status of cardiac tissue in pathological settings.

(4) Kenneth Tran

Regulation of cardiac cellular bioenergetics

Kenneth Tran, Denis S. Loiselle and Edmund J. Crampin

The regulation of cardiac cellular bioenergetics is critical for maintaining normal cell function, yet the nature of this regulation is not fully understood. Different mechanisms have been proposed to explain how mitochondrial ATP production is regulated to match changing cellular energy demand while metabolite concentrations are maintained. We have developed an integrated mathematical model of cardiac cellular bioenergetics, electrophysiology, and mechanics to test whether stimulation of the dehydrogenase flux by Ca^{2+} or Pi, or stimulation of complex III by Pi can increase the rate of mitochondrial ATP production above that determined by substrate availability (ADP and Pi). Using the model, we show that, under physiological conditions the rate of mitochondrial ATP production can match varying demand through substrate availability alone; that ATP production rate is not limited by the supply of reducing equivalents in the form of NADH, as a result of Ca^{2+} or Pi activation of the dehydrogenases; and that ATP production rate is sensitive to feedback activation of complex III by Pi. We then investigate the mechanistic implications on cytosolic ion homeostasis and force production by simulating the concentrations of cytosolic Ca^{2+}, Na^{+} and K^{+}, and activity of the key ATPases, SERCA pump, Na^{+}/K^{+} pump and actin-myosin ATPase, in response to increasing cellular energy demand. We find that feedback regulation of mitochondrial complex III by Pi

improves the coupling between energy demand and mitochondrial ATP production and stabilizes cytosolic ADP and Pi concentrations. This subsequently leads to stabilized cytosolic ionic concentrations and consequentially reduced energetic cost from cellular ATPases.

Session 8: Hypothalamic dysfunction in cardiovascular disease

(1) Colin Brown

Contribution of vasopressin neurons to the development of angiotensin-dependent hypertension

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Vasopressin is a potent vasoconstrictor. Normally, vasopressin secretion is under tonic inhibition by arterial baroreceptors and increased blood pressure increases baroreflex inhibition of vasopressin secretion to return blood pressure toward resting levels. Paradoxically, vasopressin levels are elevated in some patients with established hypertension but it is not known whether elevated vasopressin levels contribute to the development of hypertension. We found that subcutaneous vasopressin V1a receptor antagonist administration blunts the increase in blood pressure in transgenic Cyp1a1-Ren2 rats with inducible angiotensin-dependent hypertension. Secretion of vasopressin from the posterior pituitary gland is determined by action potential discharge of hypothalamic vasopressin neurons. In extracellular single-unit recordings of vasopressin neurons from urethane-anaesthetised rats, vasopressin neuron firing rate was higher in hypertensive rats than in non-hypertensive rats. Intravenous α_1-adrenoreceptor agonist, phenylephrine, caused baroreflex inhibition of vasopressin neurons in non-hypertensive rats, but not in hypertensive rats. The final mediator of baroreflex inhibition of vasopressin neurons is an inhibitory GABAergic afferent input. In whole-cell patch-clamp recordings from brain slices, the $GABA_A$ receptor antagonist, bicuculline, excited vasopressin neurons from non-hypertensive rats and the $GABA_A$ receptor agonist, muscimol, excited vasopressin neurons from hypertensive rats. Taken together, our results suggest that increased vasopressin secretion contributes to the development of hypertension and is driven by reduced baroreflex inhibition of vasopressin neurons via a switch in GABA inhibition to excitation.

(2) Daryl Schwenke

Identifying the neural network that triggers sympathetic activation following acute myocardial infarction.

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Myocardial infarction (MI) triggers an adverse and sustained increase in sympathetic nerve activity (SNA) to the heart, provoking arrhythmias and is a leading factor for the high mortality rate within the ensuing hours. The mechanism(s) responsible for this adverse increase in SNA following MI

remain to be fully elucidated, although the progressive activation of various nuclei within the brain, from peripheral and central inputs, ultimately governs sympathetic output. Importantly, the interaction between different nuclei that are responsible for triggering the adverse increase in SNA has not been investigated. This study first aimed to essentially 'map' activation of key nuclei known to modulate sympathetic activation, within the very early stages following acute MI, using Fos expression as a marker of neuronal activation. The results showed that, even within 90 min of an acute MI (induced by ligation of the LAD coronary artery), a plethora of nuclei within the CNS were clearly activated, such as the supraoptic nucleus, subfornical organ, nucleus tractus solatarius and rostral ventral lateral medulla. In particular, the paraventricular nucleus (PVN), showed a significantly greater magnitude of neuronal activation, compared to other nuclei, following MI. Given that pre-autonomic neurons of the PVN are located within the parvocellular division of the PVN (as opposed to the magnocellular division), and that distinct populations of phenotypically different neurons exist within the PVN, the second aim of this study was to identify the primary pre-autonomic neuronal phenotype, using double label immunohistochemistry, that accounts for PVN activation following acute MI. The results indicate that activation of PVN oxytocin neurons, rather than vasopressin neurons, play a predominant role in sympathetic activation, at least within the early stages of the MI event. These novel results provide important insight into the neuronal network that is activated and triggers sympathetic activation in the early stages following acute MI.

(3) Elisabeth Lambert

Elevated sympathetic tone as an etiological factor of cardiometabolic disease

Elisabeth A Lambert

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The sympathetic nervous system (SNS) plays a key role in both cardiovascular and metabolic regulation hence disturbances in SNS regulation are likely to impact on both cardiovascular and metabolic health. With excess adiposity, in particular when visceral fat accumulation is present, sympathetic activation commonly occurs. The cause and extent of sympathetic activation in obesity may be attributed to factors such as the release of adipokines from the adipose tissue, the existence of sleep apnoea, components of the metabolic syndrome, distribution and type of fat and underlying stress. Sympathetic activation may lead or further aggravate the elevation of blood pressure and end organ damage including vascular, cardiac and renal impairment. Weight loss achieved by either lifestyle changes including diet and exercise programs or bariatric surgery considerably decrease sympathetic tone, improve the cardiovascular and metabolic profile of obese subjects and decrease their cardiovascular risk. Whether some of the beneficial effects are due to an inhibitory effect on sympathetic nervous activity is not known. Given the lack of success in sustaining long term weight loss after diet or exercise the development of interventions and strategies that optimise weight loss but also limit obesity related cardio-metabolic disease development and progression is vital. Pharmacological and device based approaches to directly or indirectly target the activation of the SNS may offer some benefit in reducing the cardio-metabolic consequences of obesity.

(4) Carolyn Barrett

Sympathetic innervation of the heart in heart failure: role of the renal nerves

Carolyn J. Barrett, Sarah-Jane Guild, T. Michael Loftus, Max I. Pinkham
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Over-activity of the sympathetic nervous system is recognised as a major driver in the development of heart failure. Chronically elevated cardiac sympathetic tone drives cardiac tissue remodelling, places further stress on an increasingly overworked heart and raises the risk of fatal arrhythmias. Elevations in renal sympathetic activity contributes to the fluid retention seen in heart failure, additionally, there is growing evidence to suggest that activation of the renal nerves in heart failure results in further sympathetic drive to the heart. Our studies have examined the role the renal nerves are playing in the development of heart failure in a rat myocardial infarction induced model of heart failure. In this model direct recording of renal sympathetic activity revels that renal nerve activity increases early in the progression of the disease. We have found that removing the renal nerves resulted in less fibrosis, and normalization of both the response to beta-adrenergic blockade and sympathetic innervation of the heart. Specifically, whereas, heart failure resulted in significantly reduced expression of sympathetic and total nerves in the viable ventricular tissue, renal denervation increased this density, resulting in nerve densities comparable to that seen in rats with normal cardiac function. These studies suggest targeting the renal nerves may help to attenuate heart failure progression via preventing the adverse changes in cardiac innervation. In particular, renal denervation resulted in the conservation of sympathetic nerves in the ventricles consistent with improved contractility and reduced arrythmogenisis.

POSTER SESSION

(1) How does epicardial adipose tissue make the human heart more susceptible to atrial fibrillation?

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Atrial fibrillation (AF) is the most common cardiac arrhythmia that associated with significant cardiovascular morbidity and mortality rates. Spontaneous cardiac contraction, automaticity, is regard as a leading mechanism for developing AF. Epicardial adipose tissue (EAT) is an adipose layer located between the epicardium and visceral pericardium. Presently, EAT is considered as an emerging risk factor for developing AF. However, the interaction between EAT and the atrial myocardium which leads to cardiac automaticity is still unknown.
Therefore, this study aims to determine how EAT drives Ca^{2+} mishandling and sympathetic hyperactivity that will lead to the development of automaticity in atrial myocardium. Also, it aims to analyze the rate of fibrosis and autonomic innervations in the human right atrial tissue.
Thin muscles (trabeculae) were isolated from the right atrial appendage (RAA) obtained from obese (BMI ≥30.0 kg/m^2), overweight (BMI= 25-29.9 kg/m^2) and lean (BMI=20.0-24.9 kg/m^2) patients undergoing coronary artery bypass graft (CABG) surgery. The trabeculae were implicated for inducing the atrial automaticity during calcium overload (1-5mM) and sympathetic overload, represented by isoproterenol, a β-adrenergic agonist (10^{-8}-10^{-5}M). Moreover, the muscles were paced at 1-4Hz followed by one minute post-rest period. Any contraction within the post-rest period is defined as automaticity.
Following Ca^{2+} overload, automaticity in the human trabeculae was significantly observed only at 3mM Ca^{2+} ($p=0.03$). Whereas, unexpectedly the automaticity was only occurred at 1Hz following increased isoproterenol concentrations ($p=0.01$), even after reversing the pacing rate to (3-1Hz) ($p=0.0005$).
This study established a protocol to mimic the automaticity in the human right atrial trabeculae. In future, this study will investigate how EAT affects the atrial automaticity to increase the susceptibility to AF.

(2) O-GlcNAcylation regulates RyR2 function directly

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O-GlcNAcylation is the enzymatic addition of a sugar, O-linked β-N-Acetylglucosamine, to the serine and threonine residues of proteins, and it is abundant in diabetic conditions due to hyperglycaemia. We have recently shown that O-GlcNAcylation can indirectly increase pathological Ca^{2+} leak through the cardiac ryanodine receptor (RyR2) due to activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII). However, as RyR2 is well known to be directly regulated by other forms of serine and threonine modification (phosphorylation) this study aimed to determine whether RyR2 is directly

modified by O-GlcNAcylation and if this also alters the function of RyR2. We found that RyR2 is O-GlcNAcylated and that this modification is increased in diabetic patients. O-GlcNAcylation of RyR2 was also observed in HEK293 cells expressing RyR2 and was increased by the addition of thiamet-G (an O-GlcNAc promotor). Using the same HEK293 cells we found that high glucose increases the level of Ca^{2+} leak through RyR2 and that this effect was enhanced by thiamet-G and blunted by the O-GlcNAc inhibitor, diazo-6-oxornoleucine (DON). Intriguingly, the application of the CaMKII inhibitor, KN93, in HEK293 cells, could not completely reverse the effect of thiamet-G. Combined, these data suggest that the function of RyR2 can be directly regulated by O-GlcNAcylation, in addition to indirect regulation by CaMKII.

(3) CaMKII mediates vascular smooth muscle cell activation central to the development of atherosclerosis

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Calcium/calmodulin dependent protein kinase II (CaMKII) is an important regulator of calcium homeostasis and cell function. CaMKII activation is driven by local increases in intracellular calcium that relieves an auto-inhibitory interaction between the catalytic and regulatory domain. Under conditions of increased stress or elevated reactive oxygen species, CaMKII is subject to post-translational modifications including oxidation and phosphorylation. These modified forms of CaMKII have been linked to the development of cardiac disease. The ability of CaMKII to respond to dynamic changes in the local environment and transduce these modifications into a downstream pathological gene response make it a plausible mediator for vascular disease, where similar conditions persist. Proliferation and migration of vascular smooth muscle cells is a key event in the development of an atherosclerotic plaque, driven by heightened levels of cytokines and oxidative stress. We hypothesized that chronically active CaMKII would mediate a proliferative and migratory response in vascular smooth muscle cells (VSMCs). Using a transient transfection method, CaMKII was overexpressed in human coronary artery smooth muscle cells (HCASMCs). CaMKII overexpression led to a dramatic 15-fold ($p < 0.001$) increase in IL-6, the marker for proliferation. Furthermore, this effect was augmented under inflammatory conditions. Importantly, the effect of CaMKII on IL-6 was associated with the presence of the phosphorylated and oxidized forms of CaMKII. This study has provided early evidence for CaMKII as a potential mediator for plaque development and suggests a therapeutic potential for CaMKII inhibition in controlling the early progression of atherosclerosis.

(4) RyR2 leak and CaMKII post-translational modifications in the heart – Can this explain the altered Ca^{2+} handling in FSHD?

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The cardiac ryanodine receptor (RyR2) is responsible for releasing calcium (Ca^{2+}) from the sarcoplasmic reticulum into the cytosol in response to electrical excitation. RyR2 has the ability to be phosphorylated by two proteins, PKA (on Ser2808) and CaMKII (on Ser2814), both of which can result in excessive Ca^{2+} being released into the cytosol which can lead to disturbed muscle contraction leading to cardiac arrhythmias and induce further pathological conditions in the heart. Under normal conditions CaMKII can phosphorylate RyR2, in response to stress, to increase the release of Ca^{2+} into the cytosol. However, research has shown that CaMKII can undergo autonomous phosphorylation which results in stress independent activity leading to excessive RyR2 phosphorylation, resulting in mass Ca^{2+} leak in the heart. Research further reports that in a strongly oxidant environment CaMKII can be oxidised, which leads to the phosphorylation of RyR2 and Ca^{2+} release from the sarcoplasmic reticulum. The modifications of both CaMKII and RyR2 have been extensively researched in type 2 diabetes mellitus cardiac muscle, a disease which results in an increase in oxidative stress and inflammation. Facioscapulohumeral muscular dystrophy (FSHD) is a progressive skeletal muscle wasting disease in which Ca^{2+} handling is disturbed and skeletal muscle contraction is decreased. FSHD results in an increased amount of oxidative stress and inflammation, which may be playing a key role in altered Ca^{2+} handling. To date, the underlying mechanisms of FSHD are not well known and the research area is still in its infancy, which is why researching novel mechanisms is key to obtaining a full understanding of the disease state. We hypothesise that the modifications to CaMKII, and as a result RyR2, can explain the altered Ca^{2+} handling and decreased skeletal muscle contraction observed during FSHD. The therapeutic targets that have been researched in cardiac muscle will be reviewed to assess their potential use in skeletal muscle, in particular for FSHD.

(5) 17β-Estradiol induced calcification and altered CaMKII expression in a mouse model of atherosclerosis.

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Vascular calcification, a complication of vascular diseases, underlies a number of major adverse cardiovascular events. The calcification of atherosclerotic plaques, an indication of disease severity, results in instability and increased risk of plaque rupture. Epidemiological studies have shown an increased prevalence of adverse cardiovascular events in older postmenopausal women, exacerbated by long-term hormone replacement therapies (HRTs). The mechanisms through which HRTs contribute to adverse cardiovascular events remain unknown. We have evidence that 17β-Estradiol (E2) promotes calcification of vascular smooth muscle cells; the signaling pathways are yet to be elucidated. It is now widely accepted that some key signaling factors of bone mineralization also play a role in vascular calcification. The nodal signaling molecule calcium/calmodulin kinase II (CaMKII) has been shown to regulate these calcification pathways. This project examined the effects of E2 on plaque calcification and CaMKII expression in a murine model of atherosclerosis. Female ApoE-deficient mice with intermediate (25 weeks) or advanced (45 weeks) stage atherosclerosis were treated with E2 bi-weekly for 8 weeks. Alizarin Red staining for calcium in the innominate artery showed that E2 altered the composition of intermediate plaques by promoting calcification compared to vehicle-treated mice (mean calcified volume: $0.365 \pm 0.1\ \mu m^3$ and $0.164 \pm 0.04\ \mu m^3$, respectively; $p<0.05$). As arteries of mice with advanced plaques already showed significant

calcification, no additional morphological effects were observed in those treated with E2. Western blot data comparing CaMKII expression and activity in WT and ApoE null mice suggest increased activation of CaMKII in this model of atherosclerosis. Our results offer initial evidence that CaMKII is associated with atherosclerosis and may play a role in E2-induced plaque calcification.

(6) Changes in coronary vascular function following the progression of diabetes – assessed using Synchrotron Radiation Microangiography

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Type II diabetes has reached epidemic proportions worldwide and is associated with numerous long term health complications, in particular, diabetic heart disease. Unfortunately, the onset of diabetic heart disease begins within the early stages of diabetes. Impaired coronary blood flow in diabetes is, at least at a primary level, related to impaired glucose metabolism. Moreover, abnormal coronary endothelial reactivity is thought to be one of the earliest manifestations of vascular disease. Therefore in this study, we hypothesised that coronary blood flow impairment is a precursor for cardiac functional and structural deterioration in the diabetic db/db mouse.

We utilized advanced state-of-the-art Synchrotron Radiation microangiography, located at the SPring-8 facility, Hyogo, Japan to directly visualise and assess the coronary circulation of non-diabetic and diabetic db/db mice at 9, 18 and 24 weeks of age. Prior to angiography, echocardiography was used to assess cardiac function in all mice. Subsequently, microangiography images of the coronary vasculature were recorded before (baseline), and 5 minutes after the administration of acetylcholine and sodium nitroprusside, which were used to assess endothelial-dependent and endothelial–independent vasodilation, respectively. The vasodilatory capacity of the coronary vessels were measured and compared between non-diabetic and diabetic db/db mice. Our results showed that the functional integrity of coronary vessels was not impaired in diabetic mice at 9 and 18 weeks of age, even though cardiac dysfunction was evident in diabetic mice at 18, but not 9, weeks of age. However, severe coronary dysfunction was clearly evident by 24 weeks of age, based on i) the complete absence of any vasodilatory response to acetylcholine and ii) a poor vasodilatory response to nitroprusside.

These results suggest that coronary vascular dysfunction, although clearly evident in advanced diabetes, is uncertain to be an important precursor for the onset and progression of cardiac dysfunction in diabetes.

(7) Effect of Diabetes on Pro-apoptotic microRNA-532 in heart

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Hyperglycaemia in diabetes promotes cell death among cardiomyocytes either by apoptosis and/or necrosis. Early molecualr alterations have been shown to be accelearting this cell death process in response to the hyperglycemic conditions. MicroRNA-532 (miR-532), originally demonstrated to have oncogenic properties. Recent studies show that miR-532 could regulate apoptotic cell death. Therefore, aim of this study is to determine the differential expression of miR-532 in diabetic heart to understand its role in diabetes induced cardiomyocytes cell death.

The RNA and protein were extracted from human right atrial appendage (RAA) samples collected from patients undergoing coronary artery bypass graft surgery at Dunedin Hospital through Heart-Otago. In order to determine the changes in miR-532 during the evolution of diabetes, RNA and protein was also extracted from heart tissue of type-2 diabetic (db/db) and lean mice in age groups ranging from 8 to 32 weeks.

Quantitative RT-PCR analysis showed significant increase in the expression of miR-532 in human RAA. Interestingly, the onset of changes in miR-532 were observed at 16 weeks of age in diabetic mice when cardiac dysfunction is fully evolved. Further western blotting will be carried out to analyse changes in expression of apoptosis repressor with caspase recruitment domain (ARC), target protein for miR-532.

To determine if therapeutic modulation of miR-532 would influence the death of cardiomyocytes, adult moue cardiomyocytes (HL-1 cells) will be transfected with antagomir for miR-532 to knockdown the expression of miR-532 and a scramble sequence as a control. The transfected cells will then be cultured in either normal (5mM) or high (30mM) glucose concentrations, followed by caspase assay to determine the effect of miR-532 on apoptosis.

Positive results from this study will not only identify a novel mechanism for increased apoptosis in diabetes, but also a novel therapeutic modality to treat diabetes-induced cardiovascular disease.

(8) Role of B-type natriuretic signal peptide on AKT and ERK1/2 activity in myocardial rat ischemia

Role of B-type natriuretic signal peptide on AKT and ERK1/2 activity in myocardial rat ischemia

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B-type natriuretic peptide (BNP) is a hormone secreted by the heart in response to left ventricular hypertrophy and pressure overload. Post-translational cleavage of the BNP precursor generates a signal peptide (BNPsp) which has recently been shown to circulate in the blood. In patients with acute myocardial infarction, plasma BNPsp levels rise very early after symptom onset, suggesting BNPsp has potential as a novel biomarker for cardiac ischaemia. In addition, BNPsp demonstrates cardioprotective effects post-ischaemia. The underlying mechanisms, however, are unclear. The current study investigated the effect of BNPsp on the activation of three key kinases: ERK1, ERK2 and AKT.

Methods: Using the Langendorff isolated heart system, rat hearts underwent five treatment protocols (n=5-6 rats/group): Sham (continuous perfusion with physiological buffer alone); Control (40min ischaemia followed by 20min reperfusion with buffer alone); BNPsp (40min ischaemia followed by 20min reperfusion with buffer containing 0.3, 1 or 3nM BNPsp). The left ventricles were subsequently removed for western blot analysis of phosphorylated AKT (pAKT) and ERK1/2 (pERK1, pERK2) levels. Values are expressed as mean±SEM.

Results: Ischaemia/reperfusion (I/R) injury alone had no effect on ventricular pERK1 or pERK2 concentrations (pERK1: Control 73.08±16.50% vs Sham 71.71±10.62%; pERK2: Control 111.78±18.27% vs Sham 97.40±10.26%). However, unlike pERK2 (0.3nM 114.58±14.55%; 1nM 117.77±13.30%; 3nM 90.93±19.46%) pERK1 was significantly increased following I/R in the presence of BNPsp doses 0.3nM (108.28±15.78%, $p<0.05$) and 1nM (106.74±12.08%, $p<0.05$). BNPsp at 3nM had no effect on either pERK1 (85.32±27.44%) or pERK2 (90.93±19.46%). Ischaemia/reperfusion alone doubled the level of the pAKT (Sham 43±4.09% vs Control 79.36±5.65%, $p<0.05$), which was then reversed following perfusion with the highest concentration of BNPsp (3nM: 40.72±3.32%). The lower concentrations of BNPsp did not affect pAKT levels (0.3nM 74.18±7.62%; 1nM 78.19±4.19%).

Conclusion: Following myocardial ischaemic injury, BNPsp at low concentration promotes ERK1 activity, whereas at high concentration it abolishes I/R-induced AKT activity.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
H1	How does epicardial adipose tissue make the human heart more susceptible to atrial fibrillation?	Aram Babakr[1], Regis Lamberts[1], Pete Jones[1]	[1] Department of Physiology, University of Otago, Dunedin, NZ
H2	O-GlcNAcylation regulates RyR2 function directly	Chidinma A Okolo, Julia J McLachlan, Janet C McLay, Jeffrey R Erickson and Peter P Jones	Department of Physiology and HeartOtago, University of Otago, Dunedin, NZ
H3	CaMKII mediates vascular smooth muscle cell activation central to the development of atherosclerosis	Worthington, LP.[1], Erickson, J.R.[1], Heather, A.K.[1]	[1]Department of Physiology, University of Otago, Dunedin, NZ
H4	RyR2 leak and CaMKII post-translational modifications in the heart – Can this explain the altered Ca^{2+} handling in FSHD?	Denny, A.[1], Erickson, J.R.[1], Jones, P.P.[1], Heather, A.K.[1]	[1]Department of Physiology, University of Otago, Dunedin, NZ
H5	17β-Estradiol induced calcification and alters CaMKII expression in a mouse model of atherosclerosis.	Ebenebe, O.V [1]., Heather, A.K. [1], Erickson, J.R. [1]	[1]Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin, NZ
H6	Changes in coronary vascular function following the progression of diabetes – assessed using Synchrotron Radiation Microangiography	Wei M.Y. [1], Lew J.K.S. [1], Pearson J.T. [2], Katare R. [1], Schwenke D.O.[1]	1. Department of Physiology, Otago School of Medical Sciences, University of Otago, Dunedin, NZ 2. Department of Cardiac Physiology National Cerebral and Cardiovascular Center, Suita, Japan
H7	Effect of Diabetes on Pro-apoptotic microRNA-532 in heart	Chandrasekera .D[1], Fomison-Nurse .I[1], Rawal .S[1], Bunton .R[2], Galvin .I[2], Katare .R[1]	Department of Physiology, University of Otago School of Medical Sciences, Dunedin, NZ[1]. Department of Cardiothoracic Surgery,

			Dunedin Hospital, Dunedin, NZ[2]
H8	Role of B-type natriuretic signal peptide on AKT and ERK 1/2 activity in myocardial rat ischemia	M Bikou, P	Christchurch Heart Institute, University of Otago, NZ