Medsci Plenary Lecture #1: Circadian clocks in health and disease

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The molecular mechanism of circadian clocks in mammals is generated by a set of genes forming a transcriptional autoregulatory feedback loop. The "core clock genes" include: *Clock, Bmal1, Per1, Per2, Cry1* and *Cry2*. The discovery of "clock genes" led to the realization that circadian gene expression is widespread throughout the body and that the clock is cell autonomous. The cellular autonomy of circadian clocks has raised a number of questions concerning synchronization and coherence of rhythms at the cellular level as well as circadian organization at the systems level. The role of clocks in peripheral tissues has a number of important implications for disease.

In the circadian clock mechanism, CLOCK and BMAL1 activate the transcription of the *Period* and *Cryptochrome* genes. The PERIOD and CRYPTOCHROME proteins then feedback and repress their own transcription by interaction with CLOCK and BMAL1. In the mouse liver, CLOCK and BMAL1 interact with the regulatory regions of thousands of genes, which are both cyclically and constitutively expressed. These target genes are highly enriched for metabolic pathways and indeed all fundamental metabolic pathways in the cell are direct targets of CLOCK:BMAL1. Circadian transcription in the liver is clustered in time and this is accompanied by circadian occupancy of RNA polymerase II recruitment and initiation. These changes also lead to circadian fluctuations in histone H3 lysine4 trimethylation (H3K4me3) as well as H3 lysine9 acetylation (H3K9ac) and H3 lysine27 acetylation (H3K27ac). Thus, the circadian clock regulates global transcriptional poise and chromatin state by regulation of RNA polymerase II. A mechanistic description of the core circadian clock mechanism should promote our understanding of how the circadian clock system influences behavior, physiology as well as behavioral and metabolic disorders.

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1A.1: The β- and γ-subunit of ENaC play a modulatory role in shear force sensation

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The epithelial sodium channel (ENaC) is expressed in the kidney and vasculature and is important for blood pressure regulation. In the kidney and vasculature, ENaC is exposed to mechanical forces such as shear force (SF) caused by urine or blood flow. ENaC consists of three homologous subunits: α, β and γ and was found to respond to SF. Evidence suggests that β- and γ-ENaC alone may be involved in arterial mechanotransduction. Since little is known about the function of individual ENaC subunits and their role in SF sensation, this project aims to characterise the role of β- and γ-ENaC for SF sensation.

Human α, β and γ ENaC subunits were expressed as homomers (α, β or γ alone) or as dimers (αβ, αγ or βγ) in *Xenopus* oocytes and two-electrode voltage-clamp (TEVC) was employed to determine ENaC activity in response to SF. In oocytes expressing α-ENaC alone a 46% ± 7.4% current increase was observed in response to SF ($n = 19$, $p < 0.0001$, paired t-test), whereas homomeric β- or γ-ENaCs did not respond to SF. In dimeric αβ-ENaC channels the activation by SF was 17% ± 3% ($p < 0.0001$, $n = 19$) and in dimeric αγ-ENaC 222% ± 35% ($p < 0.0001$, $n = 17$). Dimeric βγ-ENaC did not respond to SF.

These experiments identify a modulatory role of β- and γ-ENaC, where β attenuates, and γ elevates the response to SF co-expressed with α-ENaC. Therefore, ENaC dysfunction observed in hypertension may be due to changes in β and γ-ENaC expression that could influence its activity in response to the urine or blood flow.

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1A.2: Shear force sensation of the epithelial sodium channel is facilitated via a connection to the extracellular matrix

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The epithelial sodium channel (ENaC) is expressed in the kidney and vasculature and crucial for electrolyte/fluid-homeostasis and blood pressure regulation. Abnormalities in ENaC activity are associated with hypertension. ENaC's activity is regulated by shear force (SF) (e.g. at the endothelium due to the blood flow) and we hypothesise that ENaC SF sensation relies on a connection to the extracellular matrix via glycosylated asparagines. We aimed to determine the importance of glycosylated asparagines and their attached N-linked glycans for ENaC SF sensation.

Site-directed mutagenesis combined with two-electrode voltage-clamp experiments were performed. α-ENaC subunits were stripped of their *N*-glycans by individually disrupting the glycosylation consensus sequence (NXS/T) N312NS to N312NA (Mutant: S314A) and N511YT to N511YA (Mutant: T513A). In both mutants an impaired SF activation was observed compared with wildtype α-ENaC (mean difference ± SEM SF-activation, wildtype 63 ± 10%; S314A 37 ± 13%, $P < 0.01$; T513A 28 ± 6%, $P < 0.01$, one-way ANOVA with multiple comparisons, n=20). Furthermore, the role of glycosylated asparagines for SF sensation was confirmed in experiments with the δ-subunit. While functionally related to the α-subunit (35% sequence identity), δ-ENaC is less responsive to SF and lacks the glycosylated asparagines responsible for SF sensation of α-ENaC. Insertion of asparagines corresponding to α N312 and N511, including their glycosylation consensus sequence, at the corresponding positions in the δ-subunit, generated two δ-subunit mutants (N292 and N487) with an increased activation by SF (mean difference ± SEM SF-activation, wildtype 8 ± 2%; N292 17 ± 3%, $P < 0.05$; N487 22 ± 5%, $P < 0.01$, one-way ANOVA with multiple comparisons, n=18 - 22).

These experiments confirm that glycosylated asparagines and the attached *N*-glycans in particular are crucial for SF-dependent activation of ENaC.

1A.3: Arrhythmogenic ATP based kinase inhibitors directly increase the activity of RyR2

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Spontaneous Ca^{2+} release via cardiac ryanodine receptors (RyR2) can occur under conditions of SR Ca^{2+} overload, a process termed store-overload–induced Ca^{2+} release (SOICR), and is known trigger for arrhythmia. ATP based kinase inhibitors are an increasing class of drugs used to treat cancer. Several of these compounds have been linked to arrhythmias in clinical trials, but the underlying mechanism is unknown. ATP is an agonist for RyR2, therefore we hypothesised that the arrhythmogenic nature of ATP based kinase inhibitors is due to an "ATP-like" activation of RyR2 leading to SOICR.

To explore our hypothesis we examined four ATP based kinase inhibitors; CX-4945, sunitinib, ponatinib and nilotinib. CX-4945 and sunitinib are competitive ATP inhibitors (class I) and could be expected to bind to the ATP site within RyR2, whereas ponatinib and nilotinib are allosteric ATP inhibitors (class II). We found that both CX-4945 and sunitinib significantly increased the propensity for SOICR, whereas neither class II resulted in any changed compared to vehicle control. We confirmed a direct effect of the class I inhibitor CX-4945 on RyR2 using single channel recordings. The application of CX-4945 did not increase channel open probability but did induce regular prolonged openings consistent with the observed increase in SOICR. Importantly, the application of ATP prior to CX-4945 prevented CX-4945 from altering channel function suggesting an unoccupied ATP binding site is required for the action of CX-4945.

Combined these data suggest that class I ATP based kinase inhibitors may be pro-arrhythmogenic due to the ability of these drugs to interact with the ATP binding site and trigger SOICR.

1A.4: Is high-density lipoprotein-based therapy an option for the treatment of muscle damage in Facioscapulohumeral Muscular Dystrophy?

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Facioscapulohumeral muscular dystrophy (FSHD) is an inherited myopathy due to a genetic mutation on the 4q35 chromosome. The mutation leads to loss of methylation in the 4q35 region that, in turn, is permissive for DUX4 expression, a gene that is silenced in the healthy adult population. DUX4 functions as a transcription factor, driving expression of proteins involved in oxidative stress, cell death and inflammatory response pathways. DUX4 expression in skeletal muscle increases fibre disorganisation, death, and ultimately loss of contraction capacity. At present, there is no cure for FSHD and only limited effective therapies. High-density lipoproteins (HDLs) are well-characterised for their antioxidant and anti-inflammatory properties and currently HDL-based therapies are in clinical trial for other oxidative stress-related diseases. We hypothesised that HDL treatment may be effective against DUX4-induced oxidative stress in skeletal muscle. To test this hypothesis, C2C12 mouse skeletal cells were transfected with a DUX4 expression vector or control vector for 48 hours to induce an oxidative stress, FSHD-like phenotype. For the final 4 hours, the cells were treated with HDL particles (32µM, the physiologic circulating concentration) or vehicle (Milli-Q). DUX4 was highly expressed in transfected cells, with no expression in control transfected cells (determined by mRNA and western blot analysis) and it was this expression of DUX4 which induced the oxidative stress phenotype. The levels of oxidative stress were shown to be significantly reduced by 91.4% (P<0.0001) with HDL treatment. In keeping with reduced oxidative stress, HDL treatment also decreased cell death significantly by 75.5% (P<0.05). Furthermore, DUX4 expression resulted in a disrupted myotube formation, and this was also improved following HDL treatment. In conclusion, our early results *in vitro* support that HDL is a potential therapy for FSHD, thus a follow up *in vivo* animal experiment is now warranted.

1A.5: The role of CaMKII in atherosclerotic plaque calcification in the ApoE-null mouse

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Vascular calcification, a complication of vascular diseases, underlies a number of major adverse cardiovascular events. The calcification of atherosclerotic plaques typically occurs during disease progression and results in instability and increased risk of plaque rupture. A number of inflammatory stimuli and osteogenic regulators driving plaque calcification have been identified. Yet atherosclerotic calcification remains a major cause of plaque rupture, thrombus formation and disease complication leading to poor prognosis and survival rate. Further understanding of these mechanisms is required to identify potential targets for therapies. Key signalling factors that modulate bone mineralization, including the nodal signalling molecule calcium/calmodulin kinase II (CaMKII), potentially underlie vascular calcification. Thus, we hypothesized that systemic inhibition of CaMKII would decrease plaque calcification in a mouse model of atherosclerosis ($ApoE^{-/-}$). Our data shows that, compared to age-matched wild-type (WT) animals, CaMKII expression was elevated in $ApoE^{-/-}$ mice with more advanced (54-weeks) atherosclerosis. Pharmacological inhibition of CaMKII using KN-93 in $ApoE^{-/-}$ mice reduced the incidence of calcification at 25-weeks from 57% (KN-92 control) to 25% (KN-93). Notably, this was associated with a trend towards reduced calcification area (KN-92 control: 28.81mm^2 vs. KN-93: 3.75mm^2). These results suggest that CaMKII is associated with atherosclerosis and may be involved in the pathogenesis of plaque calcification

1A.6: High-intensity interval exercise attenuates cardiac dysfunction and restores cardiac microRNA-126/SPRED-1/VEGF pathway in type-2 diabetic mice

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Impaired myocardial angiogenic response leads to poor cardiac perfusion, ultimately contributing to cardiac dysfunction. This has been attributed to the dysregulation of microRNA-126/SPRED-1/VEGF axis, a pro-angiogenic pathway. Therefore, restoration of microRNA-126 could be a potential therapeutic strategy. High-intensity interval exercise (HIIE) appears to be an effective intervention for cardiovascular disease. However, the molecular mechanism mediating the cardioprotection following HIIE still remains unknown.

To assess the effects of HIIE on the modulation of microRNA-126/SPRED-1/VEGF axis and myocardial angiogenic response in type-2 diabetic mice.

16-week old type-2 diabetic db/db mice with developed cardiac dysfunction were subjected to either HIIE or no exercise. Age-matched non-diabetic db/+ mice with normal cardiac function were used as controls. Mice were subjected to exercise for 5 consecutive days a week for 8 weeks. Each exercise consisted of 10 bouts of 5 minutes run interspersed by 2 minutes of rest. Cardiac function was measured every fortnight using echocardiography. Heart tissues were harvested at the end of 8 weeks of exercise to assess myocardial angiogenic properties by quantifying (i) arterioles density, total and functional capillaries using immunohistochemistry (indicated by total or perfused isolectin), (ii) microRNA-126 using real-time PCR and (iii) its direct target SPRED-1, an inhibitor of VEGF using western blot.

Diabetes induced deterioration in ejection fraction, fractional shortening and E/A ratio during the 8-week of exercise study, indicating the progression of cardiac dysfunction. Cardiac microRNA-126 was significantly downregulated by 4.3-fold ($p<0.05$). Western blot analysis confirmed a marked reduced VEGF level by 2.6-fold ($p<0.05$) and an augmented expression of SPRED-1 by 2.3-fold ($p<0.001$), a direct target of miR-126 and an inhibitor of VEGF. This is associated with a significant reduction in the number of arterioles and functional capillaries, indicating impaired angiogenesis and poor myocardial perfusion. Interestingly, HIIE increased microRNA-126 expression by 14-fold ($p<0.01$) and normalized SPRED-1 protein in diabetic heart. This is associated with a restoration of VEGF protein ($p<0.001$). Restoration of proangiogenic pathway is further confirmed by the increase of arteriole and functional capilary densities in the diabetic heart.

HIIE attenuated myocardial angiogenic properties in diabetes and reversed cardiac dysfunction. These functional changes might occur in part due to the restoration of cardiac microRNA-126/SPRED-1/VEGF pathway. This study also strongly advocates active lifestyle as a therapeutic strategy for metabolic-related cardiovascular diseases.

1A.7: Effect of chronic inflammation on circadian rhythms in the preterm fetus

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Circadian rhythms are important regulators of physiological activity, but little is known about fetal patterns, particularly during preterm life, and whether they can be altered by adverse events such as exposure to infection/inflammation. Our objective was to determine the effect of inflammation induced by low-dose lipopolysaccharide (LPS) on circadian rhythms of preterm fetuses.

Preterm fetal sheep (0.7 gestation) were given a saline (n=8) or low-dose LPS (n=8) infusion (ramping dose doubled/day from 200ng for five days), and studied for a further five days without infusion. Fetal heart rate (FHR), mean arterial blood pressure (MAP), and electroencephalographic activity (EEG) were continuously measured. Light period was between 06.00-18.00hrs.

In saline fetuses MAP increased 0.5-1.0mmHg during the day, plateauing ~18.00-02.00h, and then dipping (0.2-0.8mmHg) until 06.00hrs. FHR fell ~06.00-12.00h, progressively increased until 17-19.00h plateauing until 21-22.00h before falling (peak/nadir difference ~10bpm). EEG activity increased during the day (~5-10 μV), plateauing at night. LPS did not alter the cardiovascular circadian patterns or group values. However, LPS significantly reduced EEG activity by 50% by experiment end ($P<0.05$). The diurnal pattern was significantly attenuated or absent even post-LPS infusion.

Increasing MAP largely reflects fetal growth and this is the first study to show that growth appears to primarily increase only during the day, consistent with maternal nutrient intake. Further, it is the first to demonstrate that the fetus has nocturnal pressure dipping, a sign of normal cardiovascular health. These factors are not compromised by inflammation. However, brain activity was significantly altered by LPS exposure, suggesting brief inflammation may be deleterious to brain development.

1A.8: Effects of fatty acids on neuronal morphology and function – implications for Alzheimer's disease

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Diabetes and obesity are known to contribute to the development of Alzheimer's disease. A western style diet, enriched in long-chain saturated fatty acids, is regarded as a risk factor for these conditions. Even though the importance of polyunsaturated fatty acids for the maintenance of cognitive function is well known, possible implications of saturated fatty acids are less well understood. Here we investigated effects of palmitate, the most abundant saturated fatty acid in the western diet, on the morphology and function of hippocampal and cortical neurons. Furthermore, we analysed whether the unsaturated fatty acid docosahexaenoic acid (DHA) alters palmitate mediated cellular changes. Primary hippocampal and cortical neurons from Sprague Dawley pups were treated with either 200µM palmitate, 200µM DHA or a combination of both. Dual label-immunocytochemistry for the microtubules associated proteins MAP2 and Tau revealed that palmitate, but not DHA, leads to severe morphological changes in hippocampal and cortical neurons, including swelling of the cell body and blebbing in axons and dendrites severely implicating healthy cell function. These phenomena were due to a breakdown of the microtubules, as revealed by βtubulin staining. A three-dimensional analysis of the synaptic input, visualised by Synapsin1 staining, furthermore exhibited a reduction in the number of synapses after palmitate treatment. Interestingly, DHA was able to prevent all these changes, if applied simultaneously. We, furthermore, discovered that these lipids affect neuronal insulin signalling which may have direct functional implications for Alzheimer's disease.

1B.1: Nancy Sirett Memorial Lecture: Muscle, meat, marbling and medicine: the growth axis in livestock and biomedicine

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Production of meat needs to be more efficient for a sustainable future livestock industry. Our studies have shown that there is an interaction among the GH-IGF1-myostatin axis with that of gonadal steroids that has a profound effect on body growth and composition.

IGF1 and myostatin have opposing roles in regulating body growth and size of skeletal muscle, with IGF1 stimulating, and myostatin inhibiting, growth. To clarify their roles, we crossed myostatin null (*Mstn*$^{-/-}$) mice with mice over-expressing *Igf1* in skeletal muscle (*Igf1*$^{+}$) to generate six genotypes of male and female mice; wild-type, *Mstn*$^{+/-}$, *Mstn*$^{-/-}$, *Igf1*$^{+}$, *Mstn*$^{+/-}$:*Igf1*$^{+}$ and *Mstn*$^{-/-}$:*Igf1*$^{+}$. We found that myostatin regulated the number, while IGF1 regulated the size of myofibres. Over-expression of *Igf1* increased the mass of mixed fiber type muscles by 19% over wild-type, 33% over *Mstn*$^{+/-}$ and 49% over *Mstn*$^{-/-}$ (P<0.001). By contrast, the mass of the perigonadal fat pad was correspondingly reduced with removal of *Mstn* and addition of *Igf1*. The abundance of Akt and rpS6 in the IGF1 pathway were increased with deletion of *Mstn*, while phosphorylation of Akt^{S473} was increased by addition of the *Igf1* transgene. However, sexual dimorphism of body and muscle size persisted. Additional studies showed that expression of SOCS2 and CIS, feedback inhibitors of GH signalling, were increased by E_2 and inhibited by testosterone (T). Both gonadectomy and E_2 increased the proportion of oxidative myofibres, which, in the livestock industry, is associated with intramuscular fat (marbling). In contrast, the absence of myostatin, and presence of IGF1 and T increased the proportion of fast-twitch myofibres to produce a lean phenotype.

1B.2: Discovery and development of small molecule antagonists inhibiting the growth hormone receptor

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An increasing number of studies implicates the growth hormone (GH)/IGF1 axis in cancer and microvascular diseases associated with diabetes. Consequently, there has been a greater imperative to develop therapeutic strategies to antagonise GH function in pathological conditions. In order to discover and develop small molecule inhibitors of the GH receptor (GHR), a discovery pipeline comprised of molecule discovery (virtual screening), target engagement (surface plasmon resonance), target modulation (phospho-STAT5 AlphaScreen), functional activation in cells, in addition to counter-screens for cytotoxicity and off target engagement, was established that can identify functional, selective GHR inhibitors. A virtual drug screening discovery platform was used to screen a 115,000 lead-like compound library. A pilot library of 2045 compounds was designed and screened in primary and secondary screening assays. The top 25 inhibitory compounds were selected and the IC50 against GH-stimulated cell growth determined in a BA/F3 cell viability assay. Counter-screening was carried out in BA/F3 cells using the related cytokine interleukin 3 (IL3) or in T47D cells which do not respond to GHR antagonism. Compound cytotoxicity was determined using a lactase dehydrogenase assay. Through this evaluation we identified 3 compounds which inhibited GH and IL3-stimulated cell growth but had minimal effects on T47D cells. An AlphaScreen assay, which measures STAT5 phosphorylation, was used to assess inhibition of GHR signalling. Compound SN39208 significantly inhibited STAT5 phosphorylation and was shown to interact with the GHR. Cheminformatics and molecular docking was applied to identify other compounds related by substructure features to the active molecule and further investigation and improvement of these compounds is currently in progress.

1B.3: Melanocortin hormones regulate C57BL/6 mouse gut microbiota

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Melanocortin hormones are required for body weight regulation[1]. These hormones are known to regulate fat mass (exacerbated with high-fat diet), but little is known about the underlying molecular mechanisms. The gut microbiome is associated with obesity[2]. We hypothesised that melanocortin hormone regulation of gut microbiome contributes to body weight and fat mass regulation. To investigate this, we collected cecum samples from male and female wild type (WT) and homozygous (HOM) genetically modified mice that lack two melanocortin hormones. Mice were fed either low-fat (LF) or high-fat (HF) diet at weaning and euthanised at 19 weeks. Genomic DNA was extracted from cecum using Qiagen Fast Stool DNA kit. NZGL sequenced the 16S rRNA genes. Metagenomic analyses on the resulting data were conducted using QIIME. Microbiota α-diversity (within sampe diveristy) and β-diversity (between sample diversity) and relative abundances were analysed for eight male or female communities (n=7-8 mice/community) independently, each having either genotype or diet as a variant.

Our data show clear distinctions in the microbiota principal co-ordinate analysis (PCoA). Female HF WT or HF HOM samples cluster together and are significantly well-separated from the female LF WT or LF HOM samples (p=0.01). Diet alters microbiota composition of male WT but not HOM mice (p=0.01). Female WT samples cluster together and are significantly well-separated from female HOM mice regardless of whether they are fed LF or HF diet (p=0.01). Male WT samples cluster together and are signficantly well-separated from male HOM samples, but only when mice are fed LF diet (p=0.01).

In summary, melanocortin hormones and diet independently alter female gut microbiota composition, while melanocortin hormones alter male gut microbiota composition but only when mice are fed LF diet. Diet alters male WT but not HOM gut microbiota. It remains to be resolved whether melanocortin hormone regulation of gut microbiota causes obesity.

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1B.4: Prolactin-induced suppression of acute running wheel activity in female mice

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Prolactin has been implicated in a broad range of functions in the brain, and it has been proposed that many of these functions are involved in adaptive changes seen in the mother during pregnancy to prepare her for lactation. In particular, prolactin plays a role in energy homeostasis and evidence from rats indicates that prolactin can stimulate food intake and increase body weight, consistent with a role for prolactin and its pregnancy-specific homologue placental lactogen in promoting positive energy balance during pregnancy. We have recently observed a link between prolactin and running wheel activity; male mice with prolactin-receptors deleted from RIP-cre neurons and the pancreas have increased voluntary activity on a running wheel[1]. These data suggest that prolactin may suppress activity and thus prolactin-induced reductions in activity and potentially energy expenditure may contribute to inducing a state of positive energy balance. The aim of the current study is to determine if prolactin can influcence acute running wheel behaviour in non-pregnant mice. Female C57BL6/J mice that had been housed with access to a running wheel for at least 4 weeks received a i.p. injection of prolactin or saline 30 minutes before the start of the dark phase on the day of diestrus. Running wheel activity was measured continuously before and after treatment. This procedure was repeated at least a week later so all mice underwent both prolactin and saline treatments in a random order. Prolactin treatment significantly decreased total dark phase running wheel distance compared to vehicle treatment. In particular, running wheel activity was significantly reduced in the first three hours after prolactin injection. These data demonstrate that prolactin can acutely suppress running wheel behaviour, consistent with the hypothesis that prolactin induction of positive energy balance during pregnancy may involve changes in voluntary activity.

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1B.5: Genetic variation in genes of the GH/IGF-1 axis in human development and disease

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The spatial organisation of chromosomes inside the nucleus of a cell is linked to gene regulation and function. Genetic variation at loci can disrupt this unique arrangement of chromosomes in space, potentially leading to changes in gene expression that contribute to disease states. However, how or if alterations in 3D genome structure contributes to disease is still not well understood.

Genes related to the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis are crucial for normal growth and metabolism. Physiological functions mediated by this axis range from bone and muscle growth, reproduction, insulin synthesis and sensitivity, lipolysis and hepatic metabolism through to immune functions and neurogenesis. Compromised GH signalling and genetic variation in these genes have been implicated in disorders such as diabetes and cancer.

We hypothesise that polymorphisms which occur within the GH/IGF-1 axis genes have the potential to impact on disease phenotypes by altering or disrupting the spatial organisation of the genome.

We have collated a database of SNPs associated with GH/IGF-1 axis identified through genome-wide association studies (GWAS), online databases and published literature. We have used a computational pipeline that incorporates GWAS data, expression Quantitative Trait Loci (eQTL) associations, and spatial chromatin connections, to identify SNPs located in intron 1 of the *IGF2BP2* gene that exhibit significant spatial connectivity and are associated with changes in gene expression in cis and trans. Currently we are determining the functional effects of these polymorphisms using CRISPR-Cas9 genome editing and relevant enhancer analysis using appropriate *in vitro* cell models.

1C.1: Novel method of recording keratometry reading by using slit scanning

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Visual impairment is one of the world's most common health problem, which has a great effect on an individual's quality of life. Inspite of the advancements in the field of ophthalmology, cost effective methods for diagnosis and treatment are still lacking; in particular in third world rural population where limited health care and technologies are available.

This paper explores the possibility of using a smart phone app to diagnose visual impairment and replace expensive and complicated instruments such as digital keratometer. An app prototype test model is designed to simulate a slit lamp that determines the refractive errors which encompass all kinds of visual impairment. A prototype test model is designed which consists of two elements, a sphere to represent healthy human cornea and an ellipsoid to represent an astigmatic eye. Five slit lights are applied and captured on the surface of the elements as images, and by applying image processing using MATLAB, the most optimal location of the pixels is determined along with the optic power of the eye (K reading).

The app prototype was tested on two patients, one with keratoconus eyes and the other with post LASIK surgery. The app prototype was used to provide a K reading for each patient. The readings were then checked via manual calculations to validate the prototype results. The findings were promising and showed a close correlation between manual calculations and the prototype.

In conclusion the prototype app was able to diagnose visual impairment to an accurate level. However further tests are required from a larger pool of patients for improvement and validation.

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1C.2: Heart sound segmentation metrics and features

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Heart sound segmentation is the process of identifying and delimiting the different stages of the cardiac cycle in a recording of heart sounds (Phonocardiogram (PCG)). There are four stages in each heart cycle; the first heart sound (S1), ventricular systole (Sys), the second heart sound (S2), and ventricular diastole (Dias). Many different signal analysis methods have been used to perform heart sound segmentation with varying degrees of success. Currently the most successful methods use probabilistic models. The current state of the art, designed by Springer et al. in 2015, uses logistic regression and a hidden semi Markov model (LR-HSMM) to identify the most likely sequence of states given a heart sound recording. In this work, metrics for assessing the performance of heart sound segmentation have been investigated. Previously the sensitivity, specificity, accuracy, and F1 score (a single harmonic of sensitivity and specificity) of algorithms in identifying the first and second heart sounds has been used to measure their performance. These metrics fail to describe situations in which the software segmentation completely fails, usually when the states are confused for each other and the segmentation essentially goes out of phase. In practice such incorrect segmentation would completely invalidate the results of any further processing and classifications steps. In this work these situations were investigated in the case of the LR-HSMM segmentation algorithm. Eight fold cross validation was performed using annotations from a simultaneously recorded electrocardiogram (ECG) signal as the gold standard. Twenty seven cases of complete segmentation failure (Sensitivity or Specificity equal to 0) were identified and investigated further. The two main factors contributing to complete segmentation failure were found to be incorrect identification of the initial state, as well as incorrect estimation of the state durations.

1C.3: Parrot-inspired therapeutic robot to improve learning and social interaction of children with autism spectrum disorder

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Increasingly more children worldwide are diagnosed with autism spectrum disorder (ASD) during recent decades, which demands extensive research for the wellbeing of this community of about 74 million people around the world. Robot-assisted therapies are becoming more popular in addressing psychological, physiological, and social needs of children with ASD. Especially the deployment of biologically-inspired robots has inspired new boundaries for robot-assisted therapies. We, in this research, aim to develop a low-cost parrot-inspired therapeutic robot to improve learning and social interaction of children with ASD.

We developed a robot through several iterations and evaluated it among children with ASD through short-term and long-term user studies. Through observation and reporting from paediatricians, child psychologists, parents, and volunteers, it was identified that the parrot-inspired robot can help improve learning and social interaction among children with ASD.

To report stress reduction, we investigated protein and alpha-amylase levels of participants using urinary and salivary samples respectively. The average protein level of children dropped from 40.31 to 17.40 after interaction with the robot. These changes were statistically significant according to related-samples Wilcoxon Signed Ranks Test ($z=-2.37$, $p<.05$). The alpha-amylase levels have reduced from a mean of 305.71 to 151.29. The test results show overall reduction in the alpha-amylase levels that was statistically significant according to related-samples Wilcoxon Signed Ranks Test ($z=-2.20$, $p<05$). Through various user studies and analysis, we conclude that the developed parrot robot could improve learning and social interaction abilities of children with ASD and significantly reduce their stress levels

1C.4: Calu-3 cell model to investigate the effect of the positive pressure on the airway surface liquid layer water content

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This study investigates the effect of positive pressure on the water content of the airway surface liquid layer (ASL). Human airway epithelial Calu-3 cells grown in air-liquid interface (ALI) on permeable supports are used as a respiratory model. To simulate positive pressure scenarios and human respiratory conditions, cell supports are placed in an environmental chamber at 37°C and 100% relative humidity and exposed to positive pressures ranging from 4 cmH2O to 20 cmH2O with pressure oscillations of ± 1 cmH2O and 10 to 30 Hz frequency. Trans-epithelial electrical resistance (TEER) is measured before and after each experiment. Control TEER values are measured at low relative humidity (< 30%) and 37°C to obtain a 0% of water content on the ASL layer. Cell layer integrity and viability are analysed after each experiment.

The results show a significant difference ($p<0.05$) between control and TEER values for different pressure values. There is a reduction of water content on the ASL layer of 38.43%, 32.29%, and 30.58% after the application of 4, 10 and 20 cmH2O, respectively. No significant differences on resulting TEER values is found between for the three pressure oscillation frequencies. We hypothesized that the application of a positive pressure on respiratory epithelium may affect the natural mucus secretion and lubrication.

1C.5: Fluid loss based refreshment point positioning in running competitions

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Long distance running is one of the most popular daily sports, through which we can keep our health or take care of our body. Millions of people around the world run for different reasons like recreation, professionalism, mental balance or fun. During running activities, different kinds of dangerous medical situations can occur like fatigue fever, hampered respiration, dehydration, cardiac arrhythmia etc.

One of the most serious medical conditions during long distance running is fluid loss, which has direct influence on performance and the health of runners. Race control manage dehydration by using refreshment points as fluid sources, but estimating an optimal number of points or positioning them in optimal way is not a simple issue for sports organisers.

This research has implemented an algorithm and modelling application that estimate how often a moderate runner should rehydrate during a given half-marathon, based on environmental attributes like outside temperature, humidity, wind force and terrain characteristics. According to this refreshment frequency, a minimal number of refreshment points can be specified. Another model has been created to estimate fluid loss parameters of runners based on refreshment frequency, environmental attributes and a given refreshment point distribution. In addition, the algorithm is able to find the theoretical optimum of distributions, which generates minimum average fluid loss during the entire competition.

This presentation will describe research to date and further investigation plans around runner classification and visualization of runner dispersion.

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1C.6: Effects of inhibition of the lens microcirculation on the optical properties of bovine lenses assessed by laser ray tracing

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With age, the optical properties of the human ocular lens progressively change, leading to presbyopia and cataract. Recent MRI studies have shown that disrupting the lens water microcirculation can significantly impact the optics[1]. The purpose of this study was to link the changes in the lens cellular physiology to changes in its refractive properties[2]. A custom-built Laser Ray Tracing (LRT) system has allowed us to quantify the effects of physiological perturbations on the lens and monitor optical changes over time. Excised lenses were incubated in artificial aqueous humour (AAH, control) or in a series of pharmacological reagents to obstruct the lens water transport: (1) high extracellular K+ to depolarize the membrane potential or (2) ouabain (AAH+0.4mM ouabain) to inhibit Na+ pump activity.

LRT scans were performed on control and physiologically perturbed lenses every hour from t=0 to 4 hours (5 scans in total) to measure changes in the lens shape, power and gradient refractive index (GRIN). The LRT system delivers 151 laser beams sequentially across the lens and captures images of the individual rays passing through the lens. Imaging was performed at four projection angles: 0^{o} (on-axis), 10^{o}, 20^{o}, and 30^{o}. The control lenses did not exhibit changes in lens shape, power or GRIN over 4 hours. However, incubating lenses in high K+ produced a significant increase in optical power and decrease in GRIN at the lens center and surface. Lenses incubated in ouabain showed an increase in power and an increase in GRIN at the lens center. The optical changes produced by bovine lenses cultured in high K+ and ouabain solutions mirror the optical changes observed in human lenses with age. More specifically, the optical power increase matches the myopic shift in vision clinically observed in patients during the onset of presbyopia and cataract.

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1C.7: The cell-environment handshake: An interaction that regulates gene expression

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It is increasingly believed that the physical microenvironment influences tumour growth. Physical cues initially sensed by the integrin receptors, are transduced to signalling pathways, possibly including those regulated by Rho GTPases and the focal adhesion system. Therefore we investigated whether the overexpression of these proteins that is found in many cancers may be due to the physical environment of the tumour.

Here we investigated how substrate topography may influence cellular functions by using a bioimprint culture model. The substrate for the cultured cells was polystyrene that was flat, had been imprinted with a concave mould of the cells or imprinted with a convex mould. The three-dimensionality of the surface topography alters the cell-surface interaction and thence regulated cellular adhesion and consequently modulation of mechanosensing proteins. We investigated three ovarian cell lines, OVCAR-5, OVCAR-8 and SKOV-3.

In this study we found that in the ovarian cell lines, bioimprinted substrate topography reduced cell size, modulated morphology and increased cell proliferation. These results therefore indicate that the physical microenvironment, as distinct from the chemical and biological components, can alter tumour growth. We observed that expression of FAK, Rho and the MAP kinase Erk, were increased on bioimprinted substrates. Thus the results are consistent with the associated pathways being involved in mechanically-regulated behaviour of these ovarian cells.

These results provide evidence that a factor, physical topography of the microenvironment, which is independent of the genetic composition of the cells, has potential to alter cancer development and may be vital to tumorigenesis.

Further, in 2D cultures, inhibition of FAK suppressed cell growth in all these three cell lines, whereas inhibition of Rho suppressed cell proliferation in SKOV-3 and OVCAR-8 but promoted cell proliferation in OVCAR-5. Because these pathway proteins are sensitive to topography the questions arises whether the physical environment alters chemoresponse.

2A.1: Hypertension is associated with increased ENaC expression in the arteries of *Cyp1a1-Ren2* rats

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The epithelial sodium channel (ENaC) mediates the entry of Na^+ into epithelial cells and plays an important role in maintaining salt homeostasis and blood pressure. Recent findings have indicated that ENaC is expressed in vascular endothelial and smooth muscle cells, where it is involved in regulation of vascular tone. Healthy vessels normally respond to changes in blood pressure to maintain the blood pressure within certain margins. However, this response is compromised in vascular diseases such as hypertension. Thus, changes in ENaC expression in blood vessels may contribute to hypertension. Therefore, the aim of this study is to examine the expression of the □-, □-, and □-ENaC subunits in different types of arteries from normotensive and hypertensive animals.

The relative amounts of □-,□□-, and □-ENaC mRNAs were determined by real-time quantitative (qRT) PCR and the abundance of proteins by Western blot. qRT-PCR analysis confirmed presence of □-,□□-, and □-ENaC transcripts in carotid, mesenteric, aorta and femoral arteries. A significant up-regulation of □-ENaC was observed in all arteries from hypertensive rats. Increased β-ENaC mRNA was found in carotid and increased □-ENaC in mesenteric arteries. The increased mRNA expression was translated in elevated □-ENaC protein abundance in carotid, aorta and mesenteric arteries.

In conclusion, ENaC subunits are expressed in arteries and we have observed an increase in expression of ENaC in hypertensive arteries. This project will improve understanding of ENaC as a new determinant, and therapeutic target in vascular pathophysiology and hypertension.

2A.2: Ghrelin deletion impairs postischaemic revascularisation

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Critical limb ischaemia (CLI) is the most severe manifestation of peripheral artery disease, characterised by chronic rest pain, skin ulcerations, and gangrene. It is a highly morbid condition, resulting in a severely diminished quality of life, and a significant risk of mortality.

The primary goal of therapy in CLI is to restore blood flow to the affected limb, which is only possible by surgery. However, up to 50% of patients are not amenable for surgery and will ultimately require amputation. Therapeutic angiogenesis has been proposed as an alternative treatment for these 'no option' patients. Unfortunately, current angiogenic biologics have limited success. Ghrelin, a 28-amino acid peptide, has been shown to be a potent inducer of reparative revascularisation in a murine model of CLI, via the modulation of pro-angiogenic microRNAs (miRs). Despite this, the role of endogenous ghrelin in reparative vascularisation, especially under conditions of CLI, and the potential mechanism of action remain unknown. Therefore, we investigated the effects of ghrelin deletion on vascular remodelling in a murine model of CLI. CLI was induced by femoral artery ligation and electrocoagulation in seven to nine-month-old ghrelin -/- and ghrelin +/+ mice. We utilised laser Doppler perfusion imaging, micro-computed tomography, whole-mount imaging of vascular casted limbs, immunohistochemical analysis, functional assessments, and quantitative Real-Time PCR, to assess the role of endogenous ghrelin in post-ischaemic revascularisation. Our preliminary data suggests ghrelin knockout mice have a significant decrease in perfusion recovery, arteriogenesis, angiogenesis, and functional measures 14 days following CLI. Our preliminary results highlight the importance of endogenous ghrelin for effective neovascularisation in CLI, and advocates the use of exogenous ghrelin as a potential novel therapeutic agent for 'no option' CLI patients.

2A.3: Dissecting the functional relevance and ontogeny of altered GABAergic circuitry in polycystic ovary syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder leading to female infertility worldwide. Although classically considered an ovarian disorder, altered brain wiring may play a central role in the pathogenesis of PCOS. To study PCOS, we use a prenatally androgenized (PNA) mouse model that mimics the hyperandrogenemia and high luteinizing hormone (LH) secretion in adulthood seen in women with PCOS. Circuit mapping in PNA mice revealed a robust increase of GABAergic inputs from the arcuate nucleus (ARN GABA) innervating gonadotropin-releasing hormone (GnRH) neurons. Although GABA is typically inhibitory, *in vitro* studies show GABA depolarizing actions in GnRH neurons. In my PhD project, I hypothesized that *in vivo* optogenetic activation of ARN GABA neurons can elicit LH secretion, a proxy of enhanced GnRH neuron activity. I also aimed to investigate when altered GABAergic wiring develops in the brain, and whether we can reverse GABA-to-GnRH neuron aberrations in PNA mice. To investigate the functional relevance of this circuit, a blue-light sensitive protein, ChETA, was targeted to ARN GABA neurons. Optogenetic activation of ARN GABA neurons (20 Hz/10 min) generated a robust LH secretion in female and male mice. In addition, I evaluated the onset of GABAergic remodeling onto GnRH neurons. My results identified that increased GABAergic contact takes place before the post-pubertal manifestation of reproductive impairments in PNA mice ($P < 0.05$). However, blockade of the androgen receptor with flutamide (25 mg/kg) rescued normal GABAergic wiring and restored reproductive cycles in adult PNA mice. To date, my results provide the first evidence of LH secretion driven by specific activation of ARN GABA neurons suggesting a potential mechanism of the increased LH levels in PCOS. In addition, I showed that altered GABAergic wiring occurs early in life in a PCOS-like condition, but plasticity is still achieved with androgen signaling blockade.

2A.4: The role of calcium calmodulin dependent protein kinase II in type 2 diabetic cardiac dysfunction

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Calmodulin---dependent protein kinase (CaMKIIδ) is a multifunctional serine--- threonine kinase shown to be up regulated in human patients and animal models of diabetes, resulting in reduced myocardial performance and linked with disturbed Ca^{2+} handling. Therefore we hypothesized that inhibition of CaMKIIδ activity would preserve myocardial contractility in the Zucker diabetic fatty rat (ZDF), a model of type 2 diabetes (T2D). 20---week old T2D ZDF (*n*=10) and control (CTRL) rats (*n*=10) underwent echocardiography to assess *in vivo* cardiac function, alongside western blots on snap frozen cardiac tissue for CaMKIIδ expression. Cardiac muscles (trabeculae) were subsequently isolated from the right ventricle and myocardial force measurements were performed in the presence of an inhibitor of CaMKIIδ activity (KN93) or a peptide analogue with no CaMKIIδ inhibitory effects (KN92), with additional experiments performed with a second inhibitor of CaMKIIδ--- AIP. Additionally, intracellular Ca^{2+} transients were measured in cardiac ventricular myocytes isolated from T2D ZDF (n=6) and CTRL rats (n=6). No signs of cardiac dysfunction were found in the ZDF diabetic rats at 20 weeks of age and no change in total expression of CaMKIIδ, but significantly increased CaMKIIδ phosphorylation. Trabeculae isolated from the diabetic ZDF rat had reduced contractile force (F_{dev} and dF/dt_{max}) across all stimulation frequencies, alongside impaired relaxation (dF/dt_{min}). Inhibition of CaMKIIδ with KN93 and AIP in the diabetic trabeculae significantly improved contractile force, and relaxation kinetics. Intracellular Ca^{2+} transients and measurements of SR Ca^{2+} load showed no difference between the nDM and DM cells or any effect of CaMKIIδ inhibition. The results indicate that CaMKIIδ activation precedes advanced heart failure in the diabetic ZDF rat model and that CaMKIIδ contributes to the functional changes in diabetic trabeculae, but this is not through alterations to Ca^{2+} flux. Therefore indicating that CaMKIIδ activation may be suppressing contractility in the diabetic myocardium through another pathway.

2A.5: The structure-function relationship in cardiac remodelling

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Heart failure (HF) occurs when cardiac output does not meet the requirements of the body. HF is the result of maladaptive cardiac remodelling which alters myocardial structure and ventricular function. Conventionally HF has been identified as impaired systolic function, however clinical studies have revealed that systole is impaired in only half of HF cases, the other half exhibiting diastolic dysfunction. Current HF treatments were developed targeting patients with systolic dysfunction and these treatments are ineffective in altering the progression of HF with diastolic dysfunction. In order to design mechanism-targeted treatment, new insights must be gained regarding the multi-scale relationships between myocardial structure and ventricular function.

This study investigates the structure-function relationship using the spontaneously hypertensive rat (SHR) as a model of HF. The SHR exhibits progressive maladaptive cardiac remodelling which is similar to human HF. A spectrum of structural and functional data was obtained from groups with different degrees of cardiac remodelling, at a range of ages. Groups of untreated SHRs were compared with both angiotensin-converting enzyme-inhibitor (ACEi) treated SHRs and Wistar-Kyoto (WKY) controls. ACEi treatment has been shown to decrease interstitial collagen and alter ventricular function. Including this treatment group provided an additional degree of cardiac remodelling.

Each animal underwent cardiac magnetic resonance imaging (MRI) and in vivo pressure measurements, which allowed for assessment of stroke work, cardiac output. Measurements of torsion were also obtained with the use of tagged MRI. A collagen-specific stain was applied and the microstructure of the myocardium and was investigated using extended-volume confocal microscopy of LV tissue blocks. The ACEi treated SHRs were observed to have a decreased LV mass and an elevated EF relative to untreated SHRs. Extended-volume confocal microscopy revealed altered microstructural architecture suggesting an underlying structural basis to the functional changes.

2B.1: Circadian rhythmicity of leptin sensitivity in the hypothalamus

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Synchronisation between biological temporal clocks and metabolic responses is crucial for the survival of most species. Here, we investigated whether leptin signalling, important for the control of energy metabolism, is modulated by circadian rhythms in the hypothalamus of mice on a control diet and whether this rhythmicity is influenced by consumption of a high-fat diet (HFD). Therefore, we examined the ability of leptin to induce leptin signalling in the arcuate nucleus of fasted mice throughout the 24-hour rhythm by immunohistochemistry. Measuring activated phospho-STAT3-immunoreactive cells after leptin injection, we found that leptin sensitivity was regulated in a circadian manner in control mice. In these mice leptin sensitivity was highest in the early morning, after lights on, with sensitivity declining throughout the light phase and increasing throughout the dark phase. Surprisingly, leptin insensitivity in HFD mice was not universal, but varied during the 24-hour rhythm, with deteriorated leptin signalling occurring only during the last half of the dark phase and the first half of the light phase compared to control animals. At all other time points, leptin sensitivity was similar to control mice. To investigate the physiological effect of this rhythmicity, we next injected leptin or vehicle in control and HFD mice either in the early morning or early evening and compared their caloric intake. Surprisingly, control mice showed decreased caloric intake after leptin injections only in the early morning, while HFD mice showed no changes at all, suggesting that control mice are sensitive to exogenous leptin exclusively during the first part of the light phase. These data provide evidence that the circadian clock plays a crucial role in the control of leptin action and whole body energy homeostasis.

2B.2: The effect of general anaesthesia on circadian rhythms in mice

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Emerging evidence suggests that general anaesthesia might be partially responsible for the sleep disruption and fatigue some patients experience after surgical interventions[1]. Animal experiments have now proved that general anaesthesia can have a profound shifting effect on the circadian clock in a range of vertebrate and invertebrate species[2]. The proposed mechanism is via anaesthetic agents acting on the expression of core circadian clock genes [3,4]. While there are a number of isolated studies examining the effect of general anaesthesia on circadian rhythms of behaviour, there is no comprehensive description of the time dependence of these effects, i.e. there is no phase response curve for the effect of general anaesthesia on behavioural rhythms in mammals. The only phase response curve for anaesthesia is in fact, in honey bees [5].

In this work we investigated the effect of isoflurane anaesthesia on the circadian activity rhythms of adult mice (C57BL/6VJU) for six hours at six different time points throughout the day (circadian times (CT) 0, 4, 8, 12, 16, 20) in constant conditions (DD). With these data a PRC was plotted summarising the shifting effect of isoflurane depending on the time of the day of administration. Our mouse PRC is a "weak" Type 1 PRC with a delay portion when isoflurane is administered between CT 9 and 13. Phase advances are elicited when the anaesthetic is administered between CT 18 and 21. The maximum phase delay was 1.19 hours when isoflurane was administered at CT 13 and the maximum phase advance was 1.54 hours at CT 21.

Our findings support previous evidence of the time dependent effect of general anaesthesia on the circadian clock. These data provide an essential tool for clinicians and researchers alike to anticipate and therefore minimise the time dependent effects of general anaesthesia on circadian rhythms of behaviour.

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2B.3: High doses of Isoflurane reduce survival in aged *Drosophila melanogaster*

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General anaesthesia has undeniable benefits for patients in need of surgery, however its potential negative impact on health has not been well addressed. The potency of anaesthesia as measured by EC_{50} is found to decrease in old people. This suggests that elderly patients may be receiving larger doses of anaesthesia than required. Studies have reported a possible linkage between deeper anaesthesia and postoperative mortality, among which elderly patients are at most risk[1].

In this study, we have employed *Drosophila* as a model system to determine the specific effect of Isoflurane anaesthesia on the fly survival at different ages. EC_{50} of Isoflurane defined by immobility in young (10-day old) and middle-aged (30-day old) male flies were 0.28% and 0.20% respectively. Six-hour of Isoflurane anaesthesia was given to each age group of flies at increasing concentrations (0.84%, 1.95%, 2.79% or 4.18%), and their survival was monitored up to 40 days post anaesthesia. Survival was plotted in Kaplan-Meier curves and analysed by log-rank tests. In young flies (n=60 per group), none of the Isoflurane concentrations used showed any noticeable impact on fly survival compared to age-matched controls treated the same but without anaesthesia (p=0.67). These results are similar to those observed in young flies treated with propofol for six hours in another study[2]. In middle-aged flies (n=70 per group), no difference in fly survival was observed between Isoflurane at low concentration (0.84%, mean survival days 21.0) and control (22.9 days, p=0.25). In contrast, a dose-dependent reduction of middle-aged fly survival was evident at higher concentrations of Isoflurane (1.95%, 2.79% and 4.18% with mean survival days of 19.1, p=0.010; 17.1, p=0.005; and 14.3, p<0.001, respectively). Our data suggest that high doses of Isoflurane anaesthesia shorten life span in aged flies but not young ones.

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2B.4: NMDA receptor subunit switch disrupts the chondrocyte-intrinsic circadian clock in osteoarthritis and causes disease-associated changes in chondrocyte phenotype

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Osteoarthritis results when chondrocytes undergo a phenotype change and begin aggressively degrading cartilage tissue. Cell-intrinsic circadian clocks are important for regulating cell phenotype. We previously reported that the chondrocyte clock is altered (higher amplitude of per2 expression, lower amplitude of bmal1 expression), in osteoarthritis. Knockdown of bmal1 in non-diseased chondrocytes induces osteoarthritis-like cell phenotype changes in vitro and cartilage loss in rodents. Why the clock is changed in osteoarthritis is unknown. *N*-methyl-D-aspartate receptors (NMDARs) are critical regulators of the central circadian clock and NMDARs are expressed by chondrocytes. We hypothesised that NMDARs regulate the chondrocyte clock.

Osteoarthritic and non-diseased primary human chondrocytes were pre-treated with a NMDAR inhibitor (MK-801) prior to clock resetting by serum-shock. In osteoarthritic chondrocytes, NMDAR inhibition restored normal expression of clock genes (reduced per2, increased bmal1) and reverted chondrocyte phenotype back to the non-diseased state. Paradoxically, NMDAR inhibition in non-diseased chondrocytes resulted in increased per2 and decreased bmal1 expression and caused cells to adopt the osteoarthritic phenotype. There are several types of NMDARs. We found non-diseased chondrocytes expressed the GluN2A but not GluN2B NMDAR subunit. In comparison, osteoarthritic chondrocytes expressed GluN2B but had substantially reduced expression of GluN2A. Treatment of osteoarthritic chondrocytes with a GluN2B-specific inhibitor restored normal clock gene expression and reverted cells to the non-diseased phenotype. In contrast, knockdown of GluN2A in non-diseased chondrocytes caused osteoarthritis-like changes in clock gene expression and in cell phenotype.

We have shown that osteoarthritic chondrocytes express different NMDAR subunits than non-diseased chondrocytes. Altered NMDAR expression in osteoarthritis causes changes to the chondrocyte clock and induces disease-associated changes in cell phenotype.

2B.5: T-tubule remodelling: a cellular pathology driven by both sides of the plasmalemma?

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Transverse(t)-tubules are invaginations of the plasma membrane that form a complex network of ducts, ~300 nm in diameter, that penetrates deep within the cardiac myocyte where they facilitate a fast and synchronous contraction across the entire cell volume. There is now a large body of evidence in animal models and humans demonstrating pathological distortion of t-tubule structure has causative role in the loss of myocyte contractility that underpins many forms of heart failure. Investigations into the molecular mechanisms of pathological t-tubule remodelling to date have focused on proteins residing in the intracellular aspect of t-tubule membrane that form linkages between the membrane and myocyte cytoskeleton. In this talk, we review evidence that mechanisms of t-tubule remodelling are not limited to the intracellular side. Our recent data has demonstrated that collagen is integral part of the t-tubule network and that it increases within the tubules in heart failure suggesting fibrotic mechanism could drive cardiac junctional remodelling[1]. We examine the evidence that the linkages between the extracellular matrix, t-tubule membrane, and the cellular cytoskeleton should be considered as whole when investigating the mechanisms of t-tubule pathology in the failing heart. This talk summarises a review soon to be published in the journal Biophysical Reviews[2].

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2B.6: Prenatal formation of early t-tubules in the sheep heart

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Cardiomyocytes in the adult mammalian ventricle contain invaginations of the plasma membrane known as transverse (t)-tubules. These structures have been shown to be essential for promoting synchronised excitation-contraction (EC) coupling throughout the cell, which is vital for cardiac function. The t-tubules form a close association with the sarcoplasmic reticulum (SR) to allow the formation of junctions. Several key proteins involved in EC coupling are localised to the junction, including the SR calcium release channel – the ryanodine receptor (RyR). The lipophilic protein junctophilin-2 (JPH2) is also localised to the junction, where it has been implicated in the development of both the junctions and t-tubules. Previous studies have revealed that t-tubules do not begin to develop in rodents until after birth, however historical electron microscopy data suggest that this may not be the case in larger mammals, including humans.

We investigated the possibility of t-tubules in the large mammalian fetal heart, in what is, to our knowledge, the first fluorescent, target-specific study to characterise this development, focussing on the sheep. T-tubules were identified in the fetal sheep heart from 114 days gestation (term = 145 days), with the progressively increasing occurrence observed with gestational development. Further t-tubule maturation was seen after birth. Increasing intracellular JPH2 localisation accompanied the prenatal t-tubule development, which also demonstrated an increasing association with RyR. This occurred in parallel with developmental hypertrophy of the cardiomyocytes. Together, our findings indicate a significantly different temporal pattern of t-tubule development in the large mammalian heart compared to that in the rodent. This creates potential implications for human cardiac development, including future investigation of congenital heart disease.

2B.7: Organic carbon monoxide releasing molecules as cardioprotective agents

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Carbon monoxide (CO) is an endogenous cellular signalling molecule with cardioprotective anti-inflammatory, anti-apoptotic and vasodilatory properties, which have been highlighted in cell, tissue and whole animal models. The medical application of inhaled CO gas has however been hampered by concerns over the technical difficulties in controlling the systemic dose of a potentially toxic gas. We and others have addressed these concerns by producing compounds that can release controlled low doses of CO. The pharmacological benefits of a novel CO releasing molecule, oCOm-21, in hearts subjected to acute ischaemia-reperfusion (IR) injury is reported.

Hearts isolated from male Sprague-Dawley rats (270-300 g) were Langendorff perfused and haemodynamic parameters measured. Hearts were infused with either oCOm-21 (1, 3 and 10 μM, *n*=5) or inactive oCOm (DB-21; 1 & 10 μM, *n*=4) for 10 minutes prior to warm global ischaemia (30 min) and reperfusion (60 min).

Pretreatment with oCOm-21, but not DB-21, significantly attenuated IR-induced loss of left ventricular developed pressure (LVDP) and other indices of function compared to untreated controls (*n*=4) at 15 minutes reperfusion (78.95 ± 11.16 vs. 19.31 ± 5.91 mmHg, respectively, *P*<0.0001, two-way ANOVA). These findings indicate that CO is critical to oCOm-21 mediated cardioprotection. Furthermore, oCOm-21 treatment reduced apoptotic injury in ventricular myocardium following IR. This oCOm-21 mediated recovery of function was however prevented by concomitant administration of chelerythrine (1 μM, *n*=2), a potent inhibitor of protein kinase C (PKC). Western blot analysis of tissue homogenates revealed a chelerythrine-sensitive reduction in PKCε levels within the cytosolic compartment of oCOm-21 treated tissues compared to untreated controls (0.76 ± 0.06 vs. 1.098 ± 0.03 PKCε/ β-tubulin, respectively, *P*<0.05, one-way ANOVA) suggesting that cardioprotection is mediated by PKCε translocation to subcellular organelles.

Prophylactic treatment with oCOm-21 improves both functional and structural outcomes and may have therapeutic benefit in preventing acute cardiac IR injury.

2B.8: The molecular origins of amyloidogenic peptides

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Amyloid is a generic name for quarternary structure formed by extended b-sheets, that self-assembles to form fibrils. The best know example of amyloidogenic disease is Alzheimer's Disease (AD). In AD patients the amyloid-beta (Ab) peptide misfolds and aggregates into deposits in the brain associated with a sustained loss of neural tissue associated with a decline of memory and dementia. The cause of Abeta aggregation is unknown and whether the soluble protofibrils or the mature fibres are the toxic agents remains to be determined.

Host defense peptides (HDP) are components of the innate immune response and are found across most species. Many of these peptides exhibit antimicrobial activity, typically achieved via *membrane disruption* or acting on an intracellular target following *penetration* of the membrane layer. *Uperin 3.5* is a 17 amino acid peptide isolated from a small frog, *Uperoleia mjobergii,* that has antibiotic activity towards gram-positive bacteria. However, Uperin 3.5 also rapidly aggregates to form amyloid in buffer solution.[1]

Arguably one of the most challenging aspects of studying amyloid, lies in the propensity of amyloidogenic peptides to aggregate. Unlike the Ab, uperin 3.5 peptide is stable in aqueous solution and only after the addition of salts will aggregation begin. Using uperin 3.5 we have initiated a study of the action of amyloidogenic peptides towards cellular membranes. These biomaterials are composed of synthetic lipids that mimic either prokaryotic or eukaryotic membrane composition. Here we present our results on (i) the effect of uperin 3.5 aggregation state on the interaction with membranes that vary in composition, (ii) the influence of cholesterol in membrane on the activity of the uperin 3.5 and (iii) the influence and location of charged residues on the uperin 3.5, using four mutant peptides, on the interaction with the membrane layers. The method used to investigate the action of uperin 3.5 peptides towards membranes is the biophysical platform of quartz crystal microbalance (QCM). The results from this study indicate a role for cholesterol in directing membrane interactions for uperin 3.5. Thus, this study provides a mechanism for cellular loss in the brain of AD patients.

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3A.1: The role of connexin and pannexin hemichannels and purinergic receptors in perinatal ischemic brain injury

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Perinatal ischemic brain injury can occur when the blood supply to the brain is compromised before birth. The only treatment available is hypothermia, but this is only partially effective. Mechanisms underlying the spread of injury are poorly understood, but may include connexin (Cx43) and pannexin hemichannels (Px1), which release ATP, and purinergic receptors (P2X7R), which mediate potentially toxic effects of ATP. The effect of hypothermia on hemichannels and purinergic receptors is unknown.

Chronically instrumented near-term fetal sheep (0.85 gestation) were randomised to sham (n=3), ischemia+normothermia (n=4) and ischemia+hypothermia, (n=4) groups. Ischemia was induced by 30 minutes bilateral carotid artery occlusion. Hypothermia was started 90 minutes after the end of ischemia. Post-mortem was performed at 6 hours and fetal brain tissue was dissected and snap frozen for Western blotting.

At the onset of ischemia, EEG intensity was similarly reduced by 17±3 dB (ischemia+normothermia) and 21±1 dB (ischemia+hypothermia) compared to sham ($p<0.05$). At the onset of hypothermia, extradural temperature was reduced to 32.7±0.8 °C in the ischaemia+hypothermia group ($p<0.05$) compared to 39.5+0.1 °C in sham control and 39.5±0.2 °C in the ischemia+normothermia group. Preliminary data suggest that ischemia was associated with a 1.1-fold increase in Cx43 and a 1.6-fold increase in phosphorylated-Cx43 (ser368) in the white matter compared to sham control, which was attenuated by hypothermia (0.7 and 1.1-fold respectively). Ischemia was associated with a 1.5-fold increase in Px1 in the cortex, with no effect of hypothermia. Surprisingly, cortical P2X7R expression decreased to 0.6 of sham control after ischemia, but returned towards baseline levels in the ischaemia+hypothermia group (0.9 of sham control).

These data suggest that complex changes in the expression of connexin and pannexin hemichannels and purinergic receptors may contribute to the evolution of perinatal ischemic brain injury and that only some of these changes may be attenuated by therapeutic hypothermia.

3A.2: The TLR7 agonist Gardiquimod protects oligodendrocytes from damage after asphyxia in the preterm fetal sheep

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Toll-like receptors (TLRs), key regulators of innate immunity, are involved in brain injury both after infectious and non-infectious insults. While TLR activation, in particular that of TLR4, appears detrimental to the immature brain, our recent studies have identified a candidate TLR pathway for protection involving upregulation of cerebral TLR7 (1). In the present study, we examined the potential of the TLR7 agonist, Gardiquimod (GDQ) to ameliorate cell loss and improve electrophysiological recovery after acute profound asphyxia in preterm fetal sheep.

Fetal sheep at 0.7 gestation (days 103-104; term ~ 145 days) received a continuous intracerebroventricular (ICV) infusion of GDQ (total dose 1.8 mg/kg; GDQ-asphyxia, n = 5) or vehicle (vehicle-asphyxia, n = 9; vehicle sham-asphyxia, n = 9) at a rate of 11.1µl/minute for 3h commencing 1h after the end of a 25 minute umbilical cord occlusion (UCO). After 3 days recovery in utero, sheep were killed.

In the periventricular and intragyral white matter, GDQ infusion was associated with improved survival of oligodendrocytes (immature and mature [CNPase] and total oligodendrocytes [Oligo-2]) and reduced apoptosis (cleaved Caspase-3) and astroglial activation (GFAP) ($P < 0.05$). In regions of the grey matter, GDQ significantly reduced apoptosis (cleaved Caspase-3; caudate, putamen and CA1/2, CA3, CA4, DG regions of the hippocampus and thalamic nucleus) and improved neuronal survival ($P < 0.05$) in the caudate nucleus. Finally, GDQ administration was associated with more rapid early recovery of electroencephalogram power and spectral edge frequency.

In conclusion, our studies provide the first evidence that in preterm fetal sheep therapeutic manipulation through TLR7 signalling following asphyxia can reduce white matter injury and improve electrophysiological recovery, offering the potential to preserve myelination in a physiological manner.

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3A.3: Altered neural control in a microembolisation induced model of left ventricle dysfunction

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Left ventricular (LV) dysfunction can be induced via a variety of mechanisms in large animals. The microembolisation model of LV dysfunction has been used to investigate changes in heart function, however, neural control has not been examined. We hypothesized that LV dysfunction would be associated with altered neural control of the heart.

LV dysfunction was induced by infusion of microspheres (45 micron; 1.5 mls) into the circumflex or left descending coronary arteries. Three embolisations were conducted over 3 weeks. Each embolisation was associated with changes in the ST segment of the ECG lead. At the end of the embolisations, the animal was allowed 12-14 weeks for LV dysfunction to develop. Following echocardiography which indicated an ejection fraction of <50%, the animals (heart failure (HF) and control (C)) were instrumented with coronary blood flow probes.

Embolisation induced left ventricular dysfunction was associated with a decrease in MAP (HF; 74 ± 4 vs C; 90 ± 4 mmHg, $P<0.05$) and an increase in HR (HF; 115 ± 5 vs C; 98 ± 6 bpm, $P<0.05$). Also, there was a significant decrease in coronary blood flow in the HF group compared with control (71 ± 9 vs 152 ± 35 mL/min, $P<0.05$). Baroreflex control of HR was investigated using sigmoidal systolic BP-HR curves. There was a significant difference in the lower plateau between the two groups (HF; 74 ± 4 vs, C; 58 ± 7 beats min^{-1}, $P<0.05$).

Microembolization induced left ventricle dysfunction was associated with a decrease in coronary blood flow and impaired cardiac function. These findings suggest this model is appropriate to investigate neural regulation of heart function post LV dysfunction.

3A.4: The role of arcuate nucleus kisspeptin neurons in the generation of luteinising hormone pulses

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Kisspeptin signalling through its receptor Kiss1r is essential for reproductive function. Despite the dogma that the kisspeptin-expressing (KP) neurons in the arcuate nucleus (ARN) are an essential component of the gonadotropin-releasing hormone (GnRH) neuron pulse generator, the field has lacked the tools to definitely examine this hypothesis. Combining a sequential blood collection procedure with transgenic mice and the inhibitory optogenetic tools archaerhodopsin (ArchT) and halorhodopsin (halo) has allowed us to remotely and reversibly control the activity of the ARN kisspeptin neurons and probe their role in the generation of GnRH and luteinising hormone (LH) pulses. Adeno-associated viral vectors (AAVs) were injected bilaterally into the ARN of KP-cre mice to specifically and exclusively target the expression of ArchT or halo to the ARN KP neurons. During the sequential blood sampling procedure, the ARN KP neurons were illuminated with 532nm laser light via an indwelling bilateral fiberoptic cannula for 30min. Illumination with 532nm light, and not 473nm light, resulted in an inhibition in LH secretion for at least the duration of illumination in KP-cre mice expressing either ArchT or halo in ARN KP neurons. Illumination of the ARN KP neurons of wildtype mice injected with the AAVs did not alter LH secretion. Taking advantage of the strong rebound excitation of ARN^{KISS} neurons following inhibition with halorhodopsin, we found that re-setting the activity of the ARN KP neurons resulted in a re-setting of pulsatile LH secretion. These data indicate that the ARN KP neurons are critical for pulsatile secretion of GnRH and LH and likely represent the so-called “GnRH pulse generator”.

3A.5: Triennial Medal Award: Stating the obvious? Vasopressin neurons contribute to increased blood pressure during the development of hypertension

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Hypothalamic magnocellular neurons secrete vasopressin into the systemic circulation to maintain blood pressure by increasing renal water reabsorption and by vasoconstriction. When blood pressure rises, baroreflex activation normally inhibits vasopressin neurons via activation of GABAergic inputs. However, plasma vasopressin levels are paradoxically elevated in several models of hypertension and in some patients with essential hypertension, despite increased blood pressure. We have found that antagonism of vasopressin-induced vasoconstriction attenuates the increase in blood pressure in Cyp1a1-Ren2 rats with inducible angiotensin-dependent hypertension, suggesting that endogenous vasopressin contributes to the development of hypertension. We have also found that the activity of the neurons that secrete vasopressin is increased early in the development hypertension via blunted baroreflex inhibition of vasopressin neurons. Baroreflex inhibition of vasopressin neurons is mediated by inhibitory GABA interneurons and we have found that local administration of a $GABA_A$ receptor antagonist inhibits vasopressin neurons during, but not before, the onset of hypertension. Taken together, our data suggest that vasopressin exacerbates the increase in blood pressure evident early in the development hypertension and that blunted baroreflex inhibition of vasopressin neurons is underpinned by an excitatory shift in the response of these neurons to endogenous GABA signalling.

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3B.1: Does the neuropeptide RFamide related peptide-3 act on GnRH neurons to inhibit reproductive activity?

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The neuropeptide RFamide related peptide-3 (RFRP-3) exerts mostly suppressive effects on reproductive hormone secretion, although some stimulatory effects have been reported that appear to be specific to males (1). It is hypothesised that RFRP neurons (which synthesise RFRP-3) project to gonadotrophin releasing hormone (GnRH) neurons (which stimulate reproductive activity) in the hypothalamus of the brain to exert this effect, but other target cells have also been suggested for RFRP-3. This project aims to test if RFRP-3 acts directly on GnRH neurons to inhibit reproductive activity. To enable this, a new laboratory mouse line has been developed that uses Cre-LoxP transgenic technology to knock out RFRP-3 receptors (GPR147) specifically from GnRH neurons. Since all other cell type will retain normal expression of RFRP-3 receptors, any reproductive deficits will be attributable to direct RFRP-3 actions on GnRH neurons.

The reproductive endpoints measured will include: puberty onset (measured using observation of vaginal opening, vaginal cytology for reproductive cycle onset, and preputial separation); reproductive cycle length (measured using vaginal cytology) and frequency of luteinizing hormone pulses (which closely reflect GnRH pulses) in blood samples collected from the tail vein.

This project will enable the importance of direct RFRP-3 actions of GnRH neurons to be determined. If no reproductive phenotype is observed in the RFRP-GPR147 knockout mice, we will next delete the receptor from all forebrain neurons, or specifically from kisspeptin neurons.

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3B.2: Revealing the circadian patterns of CRH neuron excitability in freely behaving mice

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Corticosterone secretion in rodents is known to follow a circadian pattern: a steady increase starting mid-day with a plasma peak level at the onset of the animal's active phase and a trough prior to the inactive phase. In addition, an ultradian rhythm had been observed with near hourly corticosterone pulses. These rhythms are influenced, but not solely dependent, on the suprachiasmatic nucleus. Furthermore, a gating mechanism of the adrenal cortical clock, dependent on light input, plays an important role.

Previous experiments carried out in mice showed that corticotropin-releasing hormone (CRH) neuron activity increases when an animal experiences stress. However, no information is known about the circadian pattern of CRH neuron activity. Therefore, we plan to image CRH neuron activity in freely behaving mice during the course of the day to reveal this rhythm. This will be achieved by using a technique called fibre photometry in order to record the CRH neuron population activity. This involves the expression of a genetically encoded calcium dependent indicator (GCaMP6s) in the CRH neurons, followed by the implantation of a fibre optic cannula into the paraventricular nucleus (PVN) of the hypothalamus. This technique relies on the principle that GCaMP6s-expressing CRH neurons will emit more fluorescence when active and spiking compared to when inactive. By measuring these changes in fluorescence, we can differentiate between when the CRH cell population is active and inactive.

This current research will reveal how stress axis excitability is controlled in the brain across the course of the day and give insight into the neural circuit mechanisms underlying the circadian control of stress hormone secretion.

3B.3: Determining the physiological importance of CaMKII in alpha-adrenergic (α-AR) regulation of heart function in mice

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Physiological changes incurred in the heart due to T2DM alter proteins, including cardiac-specific CaMKII, and cause regulatory dysfunction in cardiac α_1 adrenergic signalling and pathology. Very little is known about alpha adrenergic regulation of heart function; however, α_1B receptors have recently been found to be present on 100% of ventricular cardiomyocytes, indicating that they must play an important physiological role in heart function. I aim to examine the change in α_1 adrenergic functional responsiveness in CaMKII-KO mice compared to WT mice.

My goals include:

1) setting up a functional Langendorff system for a mouse model

2) measuring the basal function of CaMKII knockout mice

3) Determine the change in α_1 adrenergic functional responsiveness in CaMKII KO hearts upon methoxamine application (α_1-specific agonist).

Findings from this initial experiment could lead to further work in diabetic hearts to determine diabetic pathophysiological dysregulation and potential treatment targets.

3B.4: Alterations in calcium handling proteins in diabetic and non-diabetic patients with or without post-operative atrial fibrillation

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Calcium handling in myocytes plays a large role in controlling the contractions within the heart. Under normal conditions, the movement of calcium into and out of myocytes controls the timing and force of contraction; however, if this process becomes deregulated, pathologies such as arrhythmias can develop. Post-operative atrial fibrillation is observed when normal atrial contractions become irregular and rapid. It occurs in a large proportion of patients undergoing open heart surgery, and is associated with increased morbidities, longer hospital stays and an increased risk of mortality. Despite the clinical implications, very little is known about the molecular mechanisms underlying the development of post-operative atrial fibrillation.

The current study aims to investigate whether underlying changes in calcium handling proteins explains why post-operative atrial fibrillation may develop in some patients and not others. To achieve this, western blotting, for a number of calcium handling proteins, including sarco/endoplasmic reticulum calcium-ATPase, calcium/calmodulin-dependent protein kinase II, and the sodium calcium exchanger was performed in human right atrial appendage samples from patients undergoing cardiopulmonary bypass surgery. This tissue was taken from both diabetic and non-diabetic patients, with or without post-operative atrial fibrillation. Preliminary results indicate that the levels of the sodium calcium exchanger decreased in diabetic patients with post-operative fibrillation, while the levels of sarco/endoplasmic reticulum calcium-ATPase are decreased in both diabetic and non-diabetic patients with post-operative atrial fibrillation. Furthermore, they indicate that calcium/calmodulin-dependent protein kinase II levels are higher in diabetic patients, while no effect of post-operative atrial filiation is seen, however further investigation into these changes is required.

Overall these results indicate that changes in calcium handling proteins may play a role in the development of atrial fibrillation after surgery, and therefore may be a novel target for preoperative treatment to prevent the development of atrial fibrillation and its related risks.

3B.5: Vasopressinergic control of RP3V kisspeptin neurons

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Mammalian fertility is governed by a neural circuit that ultimately controls the activity of gonadotropin-releasing hormone (GnRH) neurons. Release of GnRH at the median eminence results in luteinising hormone (LH) and follicle stimulating hormone secretion from the anterior pituitary (1). Prior to ovulation, there is a surge in the release of LH, due to the increased activity of afferent kisspeptin neurons in the rostral periventricular area of the third ventricle (RP3V) (2). These kisspeptin neurons are under the control of both circadian inputs from the suprachiasmatic nucleus (SCN) as well as ovarian steroids. One key circadian input to kisspeptin neurons is the vasopressin (AVP) neurons from the SCN. Recent evidence has shown that AVP increases RP3V kisspeptin neuron activity, in relation to the level of ovarian steroids (3). As such, we hypothesise that SCN AVP neurons are a key input onto RP3V kisspeptin neurons and their activation underpins the downstream activation of kisspeptin neurons and the resulting preovulatory LH surge.

In this project, we first aim to characterise the AVP inputs to kisspeptin neurons. Using fluorescent viral vectors targeted to SCN AVP neurons, we will be able to trace their neuronal projections in order to examine anatomically whether these neurons establish synaptic connections with RP3V kisspeptin neurons.

Following this, we aim to determine the functional relevance of SCN AVP neurons that contact RP3V kisspeptin neurons. Using brain slice electrophysiology and optogenetics, we will investigate how this microcircuit impacts the electrical activity of RP3V kisspeptin neurons. This will allow us to functionally assess how these AVP inputs can directly affect kisspeptin neurons and thus the activity of downstream GnRH neurons.

Together, this research will provide insight into how the SCN AVP neurons and RP3V kisspeptin neurons form a functional neural circuit for the control of the mammalian preovulatory surge.

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3B.6: The role of arcuate nucleus kisspeptin neurons in female fertility and LH pulse generation

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A key unanswered question in female reproductive neurobiology is that of how gonadotropin-releasing hormone (GnRH) neurons are regulated to produce pulsatile secretion of luteinising hormone (LH). The neuropeptide kisspeptin and its receptor, GPR-54, are potent regulators of GnRH neurons and are critical for fertility, with deletion or mutation of kisspeptin (KP) or GPR-54 causing failed puberty and infertility. The subpopulation of kisspeptin neurons within the arcuate nucleus (ARN) is proposed to be important for GnRH and LH pulse generation, however until recently it had been technically impossible to determine the relative contribution of the ARN KP neurons to estrous cyclicity and LH pulse generation. In the present experiments we employed a novel adeno-associated virus (AAV) harbouring a modified caspase to selectively ablate the ARN KP neurons. The Cre-dependent AAV caspase was injected bilaterally into the ARN of adult Kiss $Cre^{+/-}$ and wildtype female mice to examine the role of this subpopulation in the control of estrous cyclicity, and the generation of LH pulses. Changes in estrous cyclicity were monitored by daily vaginal cytology for 6 weeks following AAV injection. LH pulsatility was examined using a sequential tail-tip blood collection procedure 6-8 weeks following AAV injection in ovary-intact mice. Mice were then ovariectomised and the pulse bleeding protocol repeated two weeks later, and LH levels were determined by ultrasensitive LH ELISA. At the conclusion of the study mice were perfusion fixed with 4% paraformaldehyde, and free-floating immunocytochemistry performed for KP to quantify the level of ablation of ARN KP neurons in Kiss $Cre^{+/-}$ compared to wildtype mice. Preliminary findings demonstrate a decrease in KP immunoreactivity proximal to the AAV injection site, and some disruption to estrous cyclicity in Kiss $Cre^{+/-}$ mice.

3B.7: The effect of protein kinase C on the ryanodine receptor

Wong, Alexander

Cardiovascular disease (CVD) is the leading cause of death in the world. Arrhythmia is a type of CVD caused by spontaneous release of calcium in cardiomyocytes through the cardiac ryanodine receptor (RyR2). Diabetes has an increased risk of arrhythmia as well as an increase activation of Protein Kinase C (PKC), therefore, our study investigated the effect of PKC on RyR2. Spontaneous calcium release was induced in HEK293 cells expressing RyR2 with or without PKC in the presence and absence of a PKC activator. The percentage of cells experiencing spontaneous calcium release under each condition was compared. Overexpression of PKC resulted in no changes in the percent of cells spontaneously releasing calcium versus control. Similarly, activation of endogenous PKC using the PKC agonist, phosphatidylinositol 4,5-bisphosphate (DIC8) 10uM, resulted no change in activity. Inhibition of endogenous PKC showed an increase in the percent of cells spontaneously releasing calcium versus control. Unlike other kinases that can alter spontaneous calcium release through the RyR2, our finding shows that PKC has no effect and therefore may not be a component resulting in the risk of arrhythmias in diabetes.

3B.8: Hypothalamic control of stress and anxiety responses: role of CRH neurons and the neuropeptide RFamide related peptide-3

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Levels of the neuropeptide RFamide related peptide-3 (RFRP-3) increase within the hypothalamus in response to stressful situations, and treating laboratory rodents with RFRP-3 leads to activation of the hormonal stress axis and increased anxiety (1). This implies that RFRP neurons (which synthesise RFRP-3) are both modulated by and modulators of stress responses. It is hypothesised that RFRP neurons project to corticotrophin releasing hormone (CRH) neurons (which are well known for stimulating the hormonal stress axis) in the hypothalamus of the brain to exert this effect, but other target cells have also been suggested for RFRP-3. This project aims to test if RFRP-3 acts directly on CRH neurons to stimulate stress responses and anxiety-related behaviours. To enable this, a new laboratory mouse line has been developed that uses Cre-LoxP transgenic technology to knock out RFRP-3 receptors specifically from CRH neurons. Since all other cell type will retain normal expression of RFRP-3 receptors, any stress or anxiety responses will be attributable to direct RFRP-3 actions on CRH neurons.

The stress responses measured will include: anxiety behaviour (measured using light:dark box and elevated plus maze preference tests); depressive behaviour (measured using the forced swim duration test) and corticotrophin concentration in blood samples collected from the tail vein during acute restraint stress.

This project will enable the importance of direct RFRP-3 actions of CRH neurons for stress and anxiety responses to be determined. If no stress or anxiety phenotype is observed in the RFRP-GPR147 knockout mice, we will next delete the receptor from all forebrain neurons.

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3B.9: The ineluctable constraints of thermodynamics in the aetiology of obesity

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The paramount virtue of thermodynamics is its detail-independence. We capitalise on this feature to examine issues related to the development and reversal of obesity. Our approach is 'global', consistent with the First Law. That is, the metabolic energy provided by dietary foodstuffs has only three possible fates: (i) the performance of work (both microscopic and macroscopic), (ii) the generation of heat, or (iii) storage - overwhelmingly in the form of adipose tissue. Quantification of the energy expended while running a marathon reveals the inherent limitation of relying on exercise as a potent agency of weight-loss. This non-intuitive, and undoubtedly discouraging, result prompts examination of other avenues of heat loss. Since these, too, give only modest cause for optimism, we conclude that obesity can be prevented, or weight-loss achieved, only by reduction of food intake.

3B.10: Does epicardial adipose tissue make the human heart more susceptible to atrial fibrillation?

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Atrial fibrillation (AF) is the most common type of irregular heart rhythm or arrhythmia. Excessive epicardial adipose tissue (EAT), located between the epicardium and visceral pericardium, is regarded as an emerging factor for developing AF. EAT promotes AF through secreting paracrine factors including adipokines and metabolites. However, the functional interaction between EAT and myocardium to enhance AF is unknown. My study aimed to establish a protocol to measure AF in human cardiac muscles to understand the link between EAT and AF.

After informed consent, human right atrial appendage muscles (trabeculae, n = 21) were obtained from patients undergoing open chest surgery. The susceptibility of the trabeculae for developing spontaneous contractions (automaticity) were tested using a 1-minute rest period following three 1-minute stimulation frequencies (1, 2 and 3Hz) under two arrhythmogenic triggers: 1) increased external calcium $[Ca^{2+}]_o$ (1, 2 and 3 mM); 2) increased β-adrenergic activity (isoproterenol (ISO), 10^{-8} to 10^{-5} M). In a separate group of patients, trabeculae were again isolated and a piece of EAT from the same patient (weight = 80-100 mg, n = 21) was obtained. The superfusate of the EAT was circulated over the trabeculae for 30 minutes where after the automaticity protocol was repeated. We also collected the secretome of EAT after 24 hours culture and analysed the levels of resistin and in the superfusate of the EAT-trabeculae study using enzyme linked immunosorbent essay (ELISA).

The experiments demonstrated that increased external $[Ca^{2+}]_o$ increased the susceptibility of a proportion of the trabeculae for developing spontaneous contraction during the 3Hz-rest period and at 3 mM external $[Ca^{2+}]_o$ (42%, p = 0.02). Interestingly, the proportion of the trabeculae that developed spontaneous contractions following increased ISO was increased only during the 1Hz-rest period at 10^{-5} M ISO concentration (55%, p = 0.004). The circulation of EAT superfusate over the trabeculae increased the number of spontaneous contractions in the rest period that followed 1Hz at 1 and 3 mM external $[Ca^{2+}]_o$ (p = 0.02). The highest concentration of resistin was detected in the superfusate shortly after adding EAT to the trabeculae. Moreover, there was an average concentration of 2055 pg/ml resistin in the conditioned media of cultured EAT after adjusting for weight and volume of the samples.

This study established an automaticity model in the human right atrial trabeculae using common arrhythmogenic triggers for developing AF. Human EAT increased the susceptibility of the human cardiac muscle for spontaneous contractions, which might be due to increased release of resistin from the EAT.

3B.11: Liver-adipose tissue crosstalk as a key player in the pathogenesis of glycolipid metabolic disease and potential target of Chinese medicine

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Glucolipid Metabolic Disease (GLMD), a complex of interrelated metabolic disorders of glucose and lipid, has become one of the leading causes of public and health problem worldwide. As the metabolism process of lipid and glucose are highly coordinated under both physiological and diseased conditions, the impairment in the signals corresponding to the metabolism of either lipid or glucose represents one of major mechanisms underlying the pathogenesis of GLMD. The liver and adipose tissue are the major metabolic organs which exert metabolic functions through secreting several hormone-like proteins s. Fibroblast growth factor 21 (FGF21) and adiponectin represent the two major hormones secreted from the liver and adipose tissues, respectively. FGF21 possesses pleiotropic effects on maintaining the homeostasis of glucose and lipid metabolism majorly through inducing the expression and secretion of adiponectin. Therefore, FGF21-adiponectin axis works as the key mediator for the crosstalk between the liver and adipose tissue to exert the beneficial effects on the maintenance of the homeostasis in energy consumption. By contrast, adipose tissues secret proinflammatory adipokines, including adipocyte fatty acid-binding protein (AFABP) and lipocalin-2, which triggers inflammation within fat tissue and remote metabolic organs thereby exerting profound influence on whole-body metabolism Furthermore, AFABP and lipocalin-2 play a key role in the modulation of the pathogenesis of impairment in glucose and lipid metabolism. In addition, the circulating profile of these factors may serve as the novel biomarkers for the early detection of GLMD and potential target of Chinese Medicine.

Conclusions: The liver- and adipose tissue-derived factors with pleiotropic effects on regulating of lipid and glucose metabolism function as the key mediator for the crosstalk between these two highly active metabolic organs, thereby coordinating the initiation and development of GLMD.

3B.12: Diabetic cardiomyopathy is characterised by diastolic dysfunction linked with disturbances in cardiomyocyte glucose handling and AMPK signalling

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Diabetic cardiomyopathy is characterised by diastolic dysfunction but the underlying mechanisms remain unknown. Dysregulated cardiomyocyte glucose handling and metabolism in diabetes plays an important role in the cardiac pathology. The aim of this study was to investigate the role of disturbances in cardiac glucose handling and AMPK (key metabolic regulator) signalling in the development of diabetic cardiomyopathy.

Mice with diet-induced obesity and insulin resistance (high fat diet (HFD) vs AIN93G control, 15 weeks) were evaluated for systemic glucose tolerance, cardiac function (echocardiography), cardiac glycogen (enzymatic assay) and protein expression (immunoblot). Neonatal rat ventricular myocytes (NRVMs) were cultured in normal (5mM) or high (30mM) glucose for 24 hours with AMPK agonist treatment (AICAR, 1mM, 30 min).

HFD-fed mice exhibited increased body weight (27%, $p<0.01$) and decreased glucose tolerance. Diastolic dysfunction in HFD mice was evidenced by increased E/E' (39%, $p<0.05$) and positively correlated with body weight and glucose intolerance. Glycogen content was increased in the hearts of HFD mice (46%, $p<0.05$), associated with decreased phosphorylation of AMPK (Thr172, 23%, $p<0,05$). No changes in glycogen synthase or phosphorylase protein expression/phosphorylation were observed. NRVMs exposed to high glucose similarly exhibited increased glycogen content (36%, $p<0.05$) which was unaffected by AMPK activation via AICAR.

This study is the first to show that diastolic dysfunction correlates with cardiomyocyte glycogen overload in an obese, insulin resistant setting. These findings identify that disturbance of cardiomyocyte glucose storage plays a role in diabetic cardiopathology, and is not attenuated by activation of AMPK. Further investigation of alternative signalling pathways in this setting is now warranted.

3B.13: The pathophysiological role of microRNAs in diabetic cardiac stem cells

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Cardiac stem cells (CSCs) are known to regenerate the diseased heart. However, diabetes is known to cause a progressive loss in the number and functional efficacy of CSCs, thus posing a threat to their effectiveness in stem cell therapy. The mechanism behind this loss is unclear, however studies have shown that microRNAs (miRs) may be involved. MiRs are small, non-coding RNA molecules that regulate genes at the post-transcriptional level. We aim to investigate whether genetic manipulation of these miRs can regulate diabetic CSC function. We hypothesized that a change in expression of miRs in diabetic CSCs will correlate with their effect on CSC function.

RNA was extracted from Sca-1^+ CSCs isolated from type-2 diabetic (n=5) and non-diabetic (n=5) db/db mice. An ncounter miR expression assay was performed to evaluate the miR expression within the CSCs. Among 598 miRs evaluated, miR-329 (3.4 ± 0.6 (P=0.02)), miR-376c (3.9 ± 0.7 (P=0.01)) and miR-495 (2.3 ± 0.4 (P=0.02)) were significantly upregulated while miR-30c (0.4 ± 0.1 (P=0.02)) was significantly downregulated in diabetic CSCs compared to non-diabetic CSCs. Importantly, these miRs have established roles in stem cell function as miR-329, -376c and -495 inhibit the cell cycle whereas miR-30c has pro-survival effects. The differential expression of these miRs was further validated by RT-PCR. Mass spectrometry analysis predicted pro-apoptotic VDAC1 as a direct target for miR-30c and cell cycle regulator CDK6 as the direct target for miR-329, -376c and -495. Western blot analyses confirmed a marked increase in VDAC1 expression (3.9 ± 0.9 (P=0.01)) and a significant decrease in CDK6 expression (0.76 ± 0.1 (P=0.04)) thereby confirming the functional consequence of these miRs. Moreover, luciferase assays confirmed miR-30c to directly inhibit VDAC1. Our results suggest that altered miR expression may contribute to the reduced functional efficacy of CSCs in the diabetic heart by regulating apoptosis.

3B.14: Increased right cardiac sympathetic and parasympathetic nerve activity in type 2 diabetes

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Heart function is regulated by sympathetic and parasympathetic nervous inputs, which are unbalanced in type 2 diabetes contributing to widespread cardiac dysfunction. We examined the contribution of altered right cardiac sympathetic nerve activity (cSNA) and parasympathetic nerve activity (PSNA) to disturbed heart rate (HR) regulation in diabetes.

We directly measured right cSNA and PSNA in 20-week old male Zucker type 2 Diabetic Fatty rats (DM, n = 6–9) and their non-diabetic littermates (ND, n = 6–7). The right cardiac sympathetic and right parasympathetic vagal nerves were placed uncut over bipolar platinum recording electrodes, during pentobarbital anaethesia. Baseline cSNA and PSNA were recorded, followed by intravenous injection of β-agonist isoproterenol (1 μg/kg) before and after ganglionic blockade with hexamethonium (1 mg/kg).

Although HR was decreased, right integrated cSNA was increased in DM (ND 1.7 ± 0.4 vs DM 6.0 ± 2.1 μV/s, $P < 0.05$), accompanied by increased vagal PSNA frequency (ND 2.3 ± 1.1 vs DM 15.7 ± 5.6 Hz, $P < 0.05$). Diabetes significantly reduced β-adrenergic-induced increases in HR and integrated cSNA (ND 21.0 ± 5.9 vs DM 3.9 ± 2.9% change, $P < 0.05$). Disrupting transmission through nerve ganglia with hexamethonium indicated diabetes particularly impaired β-adrenergic-induced increases in signalling frequency from the heart to the brain (cSNA: ND 35.2 ± 11.7 vs DM -2.2 ± 14.4 Hz, $P < 0.05$; PSNA: ND 109.0 ± 37.2 vs DM 5.7 ± 56.5 Hz, $P < 0.05$).

Thus right cSNA and PSNA are both increased in DM, with lower HR suggesting dominant PSNA changes may be an underestimated therapeutic target. Reduced β-adrenergic responsiveness of cSNA and PSNA was largely attributable to rarely studied peripheral signalling. Severe impairment of autonomic regulation is likely a key contributor to cardiac dysfunction in type 2 diabetes.

M1: Activation of arcuate neuropeptide Y neurons alters luteinising hormone secretion in mice

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It is well known that the gonadotropin-releasing hormone (GnRH) neurons regulate the pulsatile secretion of luteinising hormone (LH) and follicle stimulating hormone (FSH) by the pituitary. However, the components of the GnRH neural circuit and how this circuit is altered in pathological conditions like polycystic ovary syndrome (PCOS) remain unclear. It is crucial to identify the afferent neurons that play an important role to sense and integrate humoral signals like hormones and relay this information to GnRH neurons. Recent work from our lab in a mouse model of PCOS suggests that GABAergic neurons in the arcuate nucleus of the hypothalamus (ARN) may serve as important afferent inputs to the GnRH neurons. Our finding that 1/3rd of these ARN GABA neurons co-express neuropeptide Y (NPY), has led us to investigate this subpopulation. NPY has long been implicated in the regulation of fertility, though the specific role of ARN NPY neurons in vivo in regulating GnRH neuron activity and LH release is still unknown. In the present study, we used the stimulatory Designer Receptors Exclusively Activated by Designer Drugs (DREADD) 'hM3Dq' to activate ARN NPY neurons and then measured the output of GnRH neurons in conscious mice. As expected, we found that activation of NPY neurons by injection of clozapine-N-oxide (CNO), a specific DREADD ligand, increased food intake in mice with unilateral or bilateral expression of hM3Dq-mCherry. As a readout of GnRH neuron activity, we measured LH using ELISA from serial blood samples collected every 6 minutes before and after injection of saline or CNO. Interestingly, in ovariectomised (OVX) mice, injection of CNO decreased baseline LH secretion compared to saline and slowed the pulsatility of LH secretion, which suggests a slowing of GnRH pulse generation. This study demonstrates an important role for ARN NPY neurons in the afferent regulation of GnRH neurons.

M2: Leptin receptor signalling in midbrain dopamine neurons suppresses physical activity in mice

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Leptin's hunger-suppressing and activity-promoting actions in the hypothalamus are well characterized, yet the mechanisms by which leptin modulates the midbrain dopamine (DA) system to influence feeding and activity behaviour remain unclear. A subset of midbrain DA neurons express leptin receptors (Lepr), and direct leptin administration to the midbrain reduced food intake and suppressed DA neuron firing in rats (1). Additionally, DA-specific deletion of leptin's main signalling molecule, STAT3, promoted voluntary running and locomotion in mice (2). While these findings suggest leptin directly targets DA neurons to influence feeding and activity behaviour, an indirect mechanism of action is also possible. Therfore, to determine whether direct leptin-DA signalling exerts a critical and/or sufficient influence on activity and feeding behaviour, we generated transgenic mice in which Lepr were specifically deleted from DA neurons (DA-Lepr) or exclusively expressed in DA neurons (DA+Lepr). We then compared leptin-induced STAT3 signalling (pSTAT3), weekly body weight (BW), daily food intake, and voluntary running and homecage activity in DA-Lepr vs Lepr-intact (WT) male mice and in DA+Lepr vs Lepr-deficient (NULL) male mice. As expected, DA-Lepr mice exhibited a significant reduction in pSTAT3 signalling in midbrain DA neurons vs WTs, whereas DA+Lepr mice exhibited a significant increase in DA pSTAT3 vs NULLs. While no differences in post-weaning BW were observed in WT vs DA-Lepr mice, DA+Lepr mice surprisingly weighed signficantly more than NULL mice from 4 to 11 weeks of age. With regards to activity and food intake, DA-Lepr mice exhibited significantly increased running wheel activity and food intake vs WT, but no differences in non-running activity. In contrast, DA+Lepr mice exhibited signifcantly reduced non-running activity vs NULLs, but no differences in running wheel activity or food intake. While still preliminary, it appears direct leptin-DA signaling is both required and sufficient to modulate different aspects of physical activity in mice.

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M3: Inhibition of growth hormone receptor signal transduction in a panel of cancer cell lines

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Growth hormone (GH) is essential for normal growth during childhood and puberty. However, elevated levels of GH have been observed in a variety of cancer types. Expression of GH in tumours is linked to a poorer survival outcome for breast and endometrial cancer patients. GH mediates actions through binding to a cell surface GH receptor (GHR), activating key signal transduction pathways including JAK/STAT, ERK and PI3-kinase/AKT. Furthermore, GH can activate the prolactin receptor (PRLR), and these two receptors can form receptor heteromultimers which may impact on the effectiveness of GHR antagonism. This study aimed to characterise a panel of cancer cell lines for components of GH signal transduction, and to determine responsiveness to GH and GHR antagonism.

Twelve breast, prostate, liver and colon cancer cell lines were characterised for GH-mediated signal transduction. STAT5 phosphorylation was assessed by western blot. Quantification of STAT5, ERK1/2 and AKT phosphorylation was carried out using AlphaScreen assays. A specific GHR antagonist, B2036 was used to determine which cells responded to GHR inhibition. The expression level of GH-related genes (*GH1, GH2, GHR*, *PRL* and *PRLR*) was also measured. A subset of cancer cell lines which respond strongly to GH treatment and to GHR antagonism was identified, as determined by STAT5 phosphorylation (e.g. prostate cancer cell line LNCaP). However, others exhibited a strong response to GH which was only partially inhibited by B2036 (e.g. cancer cell lines HepG2, 22Rv1 and ZR-75-1). This may be due to additional activation of the PRLR by GH in these cell lines. With the increasing interest in antagonising GHR signalling for therapeutic purposes, there will be a need for careful characterisation of the cancer cell lines used in preclinical studies. Future studies will determine the effectiveness of GHR antagonism in reducing tumour burden, as a monotherapy and in combination with radiation.

M4: Mitochondrial DNA content in oocytes from transgenic AMH over-expressing mice

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Female mice overexpressing Anti Mullerian Hormone have problems relating to early embryonic development, including blastocyst degeneration and increased miscarriage compared to wildtypes, however, the cause for these problems is unknown. We hypothesise that poor oocyte quality may be responsible for this phenotype. The mature oocyte contains the store of mitochondria needed for subsequent early embryonic development. Therefore, mitochondrial number below a certain threshold indicates a low-quality oocyte.

This project aims to investigate mtDNA content as an indicator of mitochondrial number in oocytes of AMH^{Tg} female mice compared to wildtype female mice to determine a possible reason for their infertility.

Oocytes were harvested and mitochondrial DNA was extracted by 10 rounds of freeze-thaw lysis. qPCR was then performed on AMH^{Tg} oocytes and wildtype oocytes to quantify mtDNA content between the two groups.

There was no significant difference in mtDNA copy numbers between AMH^{Tg} and wildtype oocytes ($P=0.218$). Therefore, the expansion of the mitochondrial population in developing oocytes does not appear to be defective in AMH^{Tg} mice. The developmental defects in AMH^{Tg} female dams are overt hence it will be surprising if the oocytes exhibit normal indications of quality. Future research will investigate other indicators of poor oocyte quality to determine the cause of the sub-fertility phenotype in AMH^{Tg} mice.

M5: Multi-frequency bioimpedance variations to estimate changes in arterial diameter

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Bioimpedance analysis (BIA) is a popular technique used in the monitoring of various physiological parameters like arterial oscillation, blood volume flow rate and cardiac output. It is based on the estimation of tissue volume changes inside the region of interest. BIA is based on measuring the impedance of the tissue under test which reflects the dielectric behavior of the tissue along with the associated dynamics. This technique generally finds applications as single frequency BIA (SF-BIA) in the form of impedance cardiography (ICG) and impedance plethysmography (IPG), or multi-frequency BIA (MF-BIA) in the form of impedance spectroscopy and tomography.

Existing methods of hemodynamic monitoring employ SF-BIA (such as ICG) where a single frequency current is introduced into the tissue, and the obtained output is processed to estimate parameters like stroke volume, cardiac output, and pulse wave velocity (PWV). This study aims at investigating the effect of blood flow-induced changes in the radial artery cross-section in the human forearm through MF-BIA. This offers a new approach to analyze the multi-frequency impedance response related to blood flow in the peripheral arteries and relate the impedance changes to estimate the changes in the diameter.

The basis of the bioimpedance theories underpin the study in this work to employ MF-BIA for analysing the effect of pulsatile blood flow in the human limbs. The study incorporates a numerical simulation of the forearm system which has been compared to an experimental simulation using tissue-mimicking phantom materials. Results from both have also been compared to parametric models of the system based on two different bioimpedance theories. Additionally, a pilot in-vivo study has been performed to ascertain whether the numerical, experimental and analytical models resemble reality. The results direct toward the potential of using a multi-frequency BIA approach for more accurate prediction of physiological changes in the human tissues, in this case, the blood flow.

M6: Elimination of skin-stretch induced motion artefacts from electrocardiogram signals

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Electrocardiography (ECG) is widely used in clinical practice, for example to diagnose coronary artery disease or the cause of chest pain during a stress test, while the patient is running on a treadmill. Ambulatory ECG monitoring is used for long term recording of ECG signals, while the patient carries out his/her daily activities. Artefacts in ECG are caused by the patient's movement, moving cables, interference from outside sources, electromyography (EMG) interference and electrical contact from elsewhere on the body. Most of these artefacts can be minimised by using proper electrode design and ECG circuitry. However, artefacts due to subject's movement are hard to identify and eliminate and can be easily mistaken for symptoms of arrhythmia and the physiological effects of exercise, leading to misdiagnosis and false alarms.

Skin stretch has been identified as a major source of motion artefacts in ECG signals, which arise due to the flow of current, called the 'injury current' across the epidermis. Thus, the skin is generally abraded or punctured to minimize variations in injury current. This is unpleasant and not useful for long term monitoring, as the skin regrows after 24 hours. Present motion sensing approaches to artefact reduction in ECG do not measure motion in terms of skin stretch.

The main goal of this study is to quantify and eliminate motion artefacts from ECG pertaining to skin stretch. A polymer patch electrode with Young's modulus lower than of skin has been developed to simultaneously measure ECG and skin stretch using an optical sensing technique. These signals were combined with infinitesimal strain theory to quantify skin stretch as two dimensional strains. Principal component analysis (PCA) and independent component analysis (ICA) were utilised for motion artefact removal from ECG signals.

A motion Artefact Rejection (AR) system has been developed to validate the approach implemented in this study. As this study mainly focuses on skin stretch induced artefacts, a plastic tube has been used to stretch the forearm skin of 7 subjects across the following age groups: 18–35 years (3 subjects), 36–55 years (2 subjects), and 56 years and above (2 subjects). ECG with motion artefacts were measured using CNT/PDMS electrodes and dry Ag electrodes. The reference ECG (ECG at rest) was measured from the chest using conventional Ag/AgCl electrodes. The average improvements in SNRs using PCA and ICA algorithms were found to be 4.249 dB and 9.586 dB respectively, while the average of maximum deviation from rest/reference ECG was 0.0843 for ECG with motion artefacts, 0.0702 for ECG after PCA and 0.0442 for ECG after ICA.

Both PCA and ICA algorithms also aided in removing baseline wander and high frequency noises in the cases of less or no motion artefact. The system performed well in removing artefacts generated due to EMG interference and stretching the skin perpendicular, diagonal and parallel to Langer's lines. Higher SNRs were achieved when PCA and ICA were performed by using 2D strains as motion information than when no motion information was used. In conclusion, ICA used for motion artefact reduction in ECG signals shows better performance than other techniques employing adaptive filtering, PCA and ICA.

A novel, state-of-the-art technique to identify and eliminate motion artefacts from ECG signals has been developed through this study which is feasible for practical implementations.

M7: Superimposed pressure oscillations (sipo) and their effects on acute and chronic asthmatic models

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The hyperconstriction of airway smooth muscle (ASM) is the main driving mechanism during an asthmatic attack. The airway lumen is reduced, resistance to airflow increases, and normal breathing becomes more difficult. The tissue contraction can be temporarily relieved by using bronchodilator drugs which induce relaxation of the constricted airways.

While widely used in asthma therapies, pharmacological treatments vary in their effectiveness from one subject to another, as do the side effects of long-term usage. Studies have shown that application of mechanical oscillations which are equivalent to the physiological patterns of normal breathing and deep inspirations in healthy airways can induce airway relaxation. This type of response is not observed in asthmatics.

Utilizing length oscillations in association with breathing patterns provides non-pharmacological options for augmenting treatment of the ASM hyperconstriction which is present in many respiratory diseases such as asthma. There is currently little known about the effects of applying superimposed pressure oscillations in combination with breathing patterns to healthy and asthmatic airways during an asthmatic attack.

Results from *in vitro* and *in vivo* studies of our laboratory's acute and chronic murine asthmatic models indicate that the use of super imposed pressure oscillations (SIPO) over normal breathing patterns facilitates relaxation during an induced asthmatic attack in healthy and asthmatic subjects. Oscillation patterns, physiological pressure equivalents, and their effects on key respiratory parameters are presented. Comparisons of healthy and asthmatic Lung Resistance (R_L) and Dynamic Compliance (C_{dyn}) values are used as assessments of the changes in airway responses to applied mechanical pressure oscillations.

Keywords: Mechanical oscillations, airway smooth muscle, breathing, contraction, relaxation.

M8: WITHDRAWN

M9: An optimized computational framework for estimating 3D atrial fibre orientations from contrast enhanced images

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Diffusion tensor imaging (DTI) is commonly used to estimate ventricular myofibre orientations but it is less effective for atria due to the relative thin wall. An alternative method is to use the structure tensor analysis (STA) through 3D eigen-analysis. The aim of this study was to further optimize the proposed STA and to validate this technique on multiple, widely used imaging methodologies.

Atrial data imaged using extended volume surface imaging and DTI were used. The STA approach, by modelling the 3D myofibre orientation as the direction parallel to the eigenvector paired with the smallest eigenvalue, was implemented in a custom written MATLAB package, as well as a fibre tracking approach. The impact of the key parameters of STA on accuracy of the estimated myofibre were systematically investigated, including the image resolution, the size of the Gaussian kernel for smoothing and number of voxels used in calculating the structure tensor. The ground truth of myofibre orientation used for comparison in our study was obtained using careful manual segmentation of atrial myo-bundles from the original image volume.

All key STA parameters were shown to have considerable impact upon myofibre orientation accuracy. Input image resolution was analysed over a range of $8\mu m^3$ to $256\mu m^3$ and was optimal at the size of a cardiac cell (~$64\mu m^3$). Gaussian smoothing prior to eigen-analysis reduced accuracy hence can be omitted whereas Gaussian smoothing post improved accuracy and had an optimal kernel size of 4. When calculating regional gradients at each pixel for the analysis, it was optimal to include only immediate neighbouring voxels.

In this study, we have demonstrated how the key STA parameters impact the accuracy of estimation of 3D myofibre orientations and, more importantly, we have defined optimal parameters. The STA provides a powerful alternative method for robust 3D myofibre estimation.

M10: Non-invasive measurement of isotropic myocardial stiffness in a hypertensive pig from MRE-measured displacements

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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 some forms of diastolic heart failure. Therefore, a quantitative measure of myocardial stiffness could be a valuable tool for stratifying patient groups. Magnetic resonance elastography (MRE) is a non-invasive way of measuring tissue stiffness. Shear waves are introduced into the tissue by an external mechanical driver. Then, an inversion method is used to estimate shear stiffness based on the displacement field, measured by phase contrast MRI.

The objective of this study was to test the implementation of the optimised virtual fields method (OVFM), a direct inverse method, with *in-vivo* animal MRE data. In this preliminary study, the method was implemented with experimental data from one pig pre- and post- hypertension, induced with a renal wrapping procedure. MRE images were collected at baseline (Bx), one month (M1) and two months (M2) after renal wrapping in six short axis locations. Then, the OVFM was used to estimate myocardial shear stiffness at end-diastole (ED) for each image slice.

There was no increase in mean myocardial shear stiffness from Bx (7.27 ± 6.41 kPa) to M1 (6.23 ± 6.18 kPa). However, there was a significant increase in mean myocardial ED tissue stiffness at M2 (21.71 ± 18.95 kPa). Mean ED pressure, averaged over five consecutive LV catheter pressure traces, increased from 0.397 kPa at Bx to 0.940 kPa at M1 and 1.756 kPa at M2. Despite a large variance in estimated shear values from slice to slice, the overall trend is in agreement with previous studies investigating changes in myocardial stiffness due to hypertension [1, 2]. This study is the first implementation of the OVFM with experimental MRE data *in-vivo* and will be applied to a cohort of animal studies in future work.

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M11: *In silico* simulation of the arterial spin labelling magnetic resonance imaging technique within the porcine pulmonary circulation

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Arterial spin labelling (ASL) magnetic resonance imaging (MRI) is an imaging technique used to measure perfusion. The perfusion is quantified by obtaining a difference image whereby blood is used as an endogenous tracer and alternately tagged as 'bright' and 'dark'. Two major limitations of this imaging technique when applied in the pulmonary circulation are the influence of an inversion gap (related to a wider tagging band than imaging band) and the contribution of signal from large conduit vessels to the MRI signal as this signal does not represent perfusion of the local capillary bed. Pigs are often used as an experimental model for studying lung function, therefore the goal of this study was to validate ASL MRI in a porcine lung model and to assess the effects of the inversion gap and conduit vessel signal. In addition, intensity cut-off values, to filter out conduit vessels, were assessed using the model.

We applied a previously developed model of the ASL MRI technique[1] in a pig lung vascular model[2] derived from both CT data and computational algorithms. A 1D Poiseuille-based flow model was used to predict the distribution of pulmonary blood flow through the arteries, capillaries and veins of the model. The ASL technique and resultant image through sagittal slices of the right lung in the supine posture was simulated by reproducing the MRI tagging procedure.

The inversion gap resulted in signal losses ranging from 29-67% across all sagittal slices. The contribution of conduit vessels to the total ASL signal was higher in the medial region (95%) compared to the lateral region (31%). As a result, the optimal intensity cut-off ranged from 35-50% from lateral to medial slices. After removal of large vessel signals, the ASL measurement reproduced perfusion gradients within 15% for all slices, thus validating ASL for porcine perfusion measurements.

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M12: Paraventricular nucleus kisspeptin fibres originate from kisspeptin cell bodies located in the periventricular nucleus

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The hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei contain magnocellular neurons that synthesize oxytocin, which is released into the circulation from the posterior pituitary gland in response to action potential firing. Oxytocin promotes uterine contractions during parturition and milk let-down during lactation. Central kisspeptin administration increases oxytocin neuron activity in anaesthetized late-pregnant rats (days 18 – 21 of gestation) but not in non-pregnant rats. We have unpublished immunohistochemistry data from mice that show increased kisspeptin projections to the PVN and SON in late pregnancy. Kisspeptin fibres were seen to wrap around the cell bodies and the apical dendrites of oxytocin neurons. To identify the origin of these kisspeptin fibres, we injected a retrograde tracer into the PVN of pregnant mice. The fluorescent retrobeads are taken up by axon terminals in the PVN and retrogradely transported along the axon to the cell body. Four days after PVN injection, mice were perfused with 4% paraformaldehyde and brains were processed for immunohistochemistry to identify kisspeptin cell bodies and co-localization of green fluorescent retrobeads. The periventricular nucleus of the hypothalamus showed co-expression of green retrobeads in kisspeptin cell bodies. Other kisspeptin neuron populations in the anteroventral periventricular nucleus and arcuate populations did not contain retrobeads. Taken together, these results show increased kisspeptin fibre innervation in the PVN and SON at the end of pregnancy in the mouse and that these fibres arise from kisspeptin cell bodies located in the periventricular nucleus. As yet the functional significance of the increase in kisspeptin fibres during pregnancy is unknown but it appears likely to increase the excitability of oxytocin neurons for birth.

M13: Using DREADDs to elucidate the role of AgRP neurons in the control of reproduction

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Agouti-related peptide (AgRP) neurons are thought to inhibit the activity of hypothalamic gonadotrophin-releasing hormone (GnRH) neurons which control fertility, but the nature of this role remains unclear. The activity of these neurons is modulated by metabolic hormones such as leptin, insulin and ghrelin. Because of this, these neurons act as an integrative system to convey metabolic cues to the reproductive system. Through using new 'Designer Receptors Exclusively Activated by Designer Drugs' (DREADDs) technology, we can now selectively activate and silence these AgRP neurons non-invasively *in vivo* using the synthetic ligand CNO to observe the effect of AgRP neuron activity in reproduction. To validate new mouse lines expressing excitatory (hM3Dq) or silencing (hM4Di) DREADDs under the control of Cre recombinase specifically in AgRP neurons, we chronically treated these mice with CNO administered through the drinking water (0.025 mg/ml). A pronounced body weight gain of 13.7% in response to AgRP neuronal excitation and loss of 11.5% in response to AgRP neuronal silencing was observed, which is consistent with the known orexigenic effects of these neurons. Using these validated mice, we are now evaluating reproductive activity in response to the activation or silencing of AgRP neurons. We hypothesize that increasing the tone of AgRP neurons will inhibit reproductive function, while decreasing the tone will have the opposite effect. Puberty onset, estrous cycles, mating success and LH concentrations will be evaluated in AgRP neuron excited, inhibited and non-DREADD expressing control mice (n=10/group). These experiments are currently underway. If AgRP neurons are inhibitory to reproduction, we would expect to see later puberty onset and time to mating, as well as a decreased LH concentration in the AgRP stimulated mice. AgRP silenced animals may show earlier puberty and increased reproductive function. These will reveal for the first time how AgRP neurons manipulate the HPG axis.

M14: Deciphering the role of arcuate GABA neurons in fertility regulation with chemogenetic tools in vivo

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Polycystic ovary syndrome (PCOS) is the most common infertility disorder in women worldwide. In a mouse model reflecting the clinical phenotype of hyperandrogenism, impaired steroid hormone feedback and infertility, we identified an alteration in the gonadotropin-releasing hormone (GnRH) neuronal network. Specifically, GnRH neurons receive greater synaptic input from GABA neurons (GABA-N) residing in the arcuate nucleus (ARN)1. To address the functional relevance of ARN GABA-N in the regulation of fertility, we are using the chemogenetic tool: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Using a Cre/lox approach, we expressed the designer receptor hM3Dq specifically in ARN GABA-N via stereotaxic injection into the ARN of vesicular GABA transporter (VGAT-Cre) mice. The delivery of the designer drug (CNO) to activate hM3Dq was coupled with serial tail-tip blood sampling to detect luteinizing hormone (LH) secretion, a readout for GnRH secretion, in gonadally intact male (n=12) and female (n=9) mice or ovariectomised female (n=4) mice. Using this approach, we have been able to accurately target hM3Dq to ARN GABA-N, elicit food intake, and trigger cFos expression specifically in ARN GABA-N after peripheral injection of 1.5mg/kg of CNO. To date, there is no evidence that the activation of ARN GABA-N by an acute peripheral injection of CNO elicits LH secretion. This result is in contrast with recent unpublished data from our lab showing a clear LH release driven by the activation of ARN GABA-N with optogenetic stimulation (Mauro Silva, unpublished results), suggesting that the acute activation of ARN GABA-N via DREADDs is not sufficient to trigger GnRH/LH release. Therefore, we are currently investigating whether chronic ARN GABA-N activation achieved through CNO delivery in the water over a 2 week period will impact estrous cyclicity and the LH pulse profile.

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M15: Regulation of the maternal hypothalamic-pituitary-adrenal axis by prolactin

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During pregnancy and lactation, the activity of the hypothalamic-pituitary-adrenal (HPA) axis is suppressed. The hormone prolactin may play a role in mediating this suppression as concentrations are elevated during pregnancy and lactation and prolactin has known anti-stress actions[1]. This study investigated this proposal using a mouse model. *In situ* hybridisation revealed a decrease in the number of *Crh* mRNA-expressing neurons in the hypothalamic paraventricular nucleus (PVN) of pregnant (day 18; 55.6 ± 9.0 cells per section) and lactating (day 7; 97.4 ± 4.9) mice in comparison to diestrous controls (186.8 ± 18.7; $p < 0.01$, Tukey-Kramer test; n = 6-7). Removal of the pups (24 h), and thus suckling-induced prolactin secretion, restored CRH neuron number (180.1 ± 19.7; n = 7).

To investigate the role of prolactin during pregnancy in suppressing *Crh* mRNA expression, a transgenic mouse model (*Prlr$^{lox/lox}$/CamK-Cre)* was used to conditionally delete prolactin receptors (PRLRs) from forebrain neurons[2]. Widespread reductions in phosphorylated signal transducer and activator of transcription 5 (pSTAT5) expression (marker of PRLR activation), measured by immunohistochemistry, confirmed the success of this approach, although pSTAT5 expression remained in the PVN. *Crh* mRNA expression was then characterised using *in situ* hybridisation in virgin and pregnant (day 16-18) *Prlr$^{lox/lox}$/CamK-Cre* mice and *Cre*-negative controls (n = 3-5). *Crh* mRNA-expressing cell number was significantly reduced in pregnant animals ($F(1,14) = 82.37$, $p < 0.0001$, two-way ANOVA), however, widespread deletion of PRLRs in the brain did not reverse this effect ($F(1, 14) = 0.1980$, $p = 0.6632$). These data indicate that prolactin signalling is not necessary for the suppression of the CRH neurons during pregnancy. However, the persistence of pSTAT5 in the PVN suggests PRLR deletion may have been incomplete in this nucleus. Thus, it remains possible that prolactin targets neurons in the PVN to mediate its anti-stress actions.

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M16: The effect of pregnancy-induced adaptations on glucagon like-peptide-1 receptor activation-induced stabilisation of beta-catenin in hypothalamic neurons in female rats

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Glucagon-like peptide-1 (GLP-1), a gut hormone released in response to feeding, acts in the brain where it regulates food intake and glucose homeostasis[1,2]. Recently our laboratory has shown that GLP-1 receptor activation is involved in a metabolic sensing mechanism via beta-catenin stabilisation in specific hypothalamic neurons of male rats[3]. Beta-catenin is a key partner with TCF7L2, a transcription factor that has strongly associated with diabetes type II[4], suggesting that this pathway might play an role in the central regulation of glucose homeostasis. In female rats, physiological demands during pregnancy induce a well-orchestrated range of adaptations to metabolic homeostasis to cope with the immediate and potential competing needs. Hyperphagia and increased adiposity are the strategies to feed the growing foetus and prepare for the metabolic demands of lactation[5], and there are also significant changes in glucose homeostasis with the development of insulin resistance and increased glucose-stimulated insulin secretion. The aim of the present study is to determine firstly, whether feeding-induced stabilisation of beta catenin in the hypothalamus also occurs in females, and if so, whether this response is altered during pregnancy. Male, diestrous female and pregnant rats were fasted for 24 hours, and then allowed free access to food for 0 or 1 or 2 hours. After the period of feeding, they were anaesthetised and perfusion fixed, and brains prepared for immunohistichemisty to determine the levels of beta-catenin protein in the hypothalamus. Unfixed brains were collected from additional groups of animals, then frozen and hypothalamic nuclei microdissected to measure expression of TCF/beta-catenin responsive genes using quantitative real time Polymerase Chain Reaction. Preliminary results of immunohistochemistry indicate that number of positive stained beta-catenin cells appear to increase in fed animals compared to fasted animals. These studies will further our understanding about the role of TCF/beta-catenin signalling in the brain in metabolic homeostasis.

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M17: Pregnancy-induced adaptations in glucose homeostasis in the mouse requires prolactin receptor expression in the pancreas but not the brain

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Major changes occur in maternal glucose homeostasis during pregnancy, with maternal tissues becoming progressively insulin resistant to prioritise glucose availability to the developing fetus. To ensure that the mother can continue to utilise glucose, and to prevent glucose overload in the fetus as the placenta becomes more efficient at capturing glucose in late pregnancy, maternal production of insulin is increased. This adaptive response involves proliferation of the insulin-secreting beta cells in the pancreas, together with increased sensitivity of glucose-stimulated insulin secretion. These changes in glucose homeostasis are thought to be driven by increased concentrations of prolactin/placental lactogen that are present during pregnancy. The aim of the current study was to determine the relative contribution of prolactin action at pancreas and/or the brain to these pregnancy-induced adptations in glucose regulation. Mice with either a deletion of the Prlr in the pancreas or brain were generated by crossing prolactin receptor (Prlr) flox mice with pdx-cre or ckc-cre mice respectively. Glucose tolerance, glucose-stimulated insulin secretion and beta cell fraction were then measured during pregnancy in knockout mice and controls. Pregnant mice with a specific deletion of Prlr in the pancreas had impaired glucose tolerance, suppressed glucose-stimulated insulin secretion and a lack of pregnancy-induced increase in beta-cell fraction indicating a critical role of for prolactin receptor in the pancreas during pregnancy. Pregnant mice with a specific deletion of Prlr in the brain showed normal pregnancy-induced adptations of glucose regulation indicating that prolactin action in the brain does not play a key role in changes in glucose regulation during pregnancy. Overall, these results indicate dysfunction of glucose homeostasis during pregnancy in mice with a conditional deletion of Prlr in the pancreas and emphasize the key role of a direct action of prolactin/placental lactogen in the pancreas in the adaptation of glucose homeostasis during pregnancy.

M18: Stabilization of beta-catenin in mouse hypothalamic cell lines

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Beta-catenin is a signalling molecule in the Wnt-signalling pathway, which has typically been associated with embryogenesis and tumorigenesis. More recently, new lines of evidence suggest that it may also be involved in the pathogenesis of type-2 diabetes. In its active form, beta-catenin acts together with the transcription factor T cell-specific transcription factor-7-like 2 (TCF7L2) to activate target genes of the Wnt-signalling pathway. Impairment in this signal transduction pathway in the pancreas may contribute to the development of type-2 diabetes. The role of the hypothalamus in controlling glucose homeostasis is becoming well-recognised, and we have recently found that Wnt signalling is activated in hypothalamus in response to feeding-related hormones. To investigate possible mechanisms of feeding-induced stabilization of β-catenin, we have used adult mouse hypothalamic cell lines that express the phenotype for various metabolic neuropeptides and receptors to survey a variety of potential hormone factors that can simulate the effect of feeding: forskolin, exendin-4 and MTII (an α-MSH analogue). Firstly, we measured the stabilisation of β-catenin in response to these treatments, using Western blotting. We found a significant increase in β-catenin protein levels 1h post-forskolin treatment, as well as an increase in Serine675-phosphorylated β-catenin following all treatments. Phosphorylation at this Serine675 increases the transcriptional activity and is therefore different to other sites that are involved in destabilisation. We next measured NPY and AgRP secretion after treating the cells with these hormones. Treatment with the hormones did not affect the secretion of either neuropeptide, however, after applying KCl to depolarise the cells, there was significantly greater release of both AgRP and NPY from treated cells compared with vehicle controls. These data suggest that the treatment has increased the pool of neuropeptide available for release. These results are consistent with the regulatory role of β-catenin signalling in the hypothalamus during a response to feeding.

M19: TRPV regulation of magnocellular neurosecretory cell activity in lactation

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Vasopressin and oxytocin are hormones that are produced by magnocellular neurosecretory cells in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus. A major role of vasopressin is to control body water balance. Under normal physiological conditions, vasopressin regulates body fluid balance via transient receptor potential vanilloid (TRPV) channels. During lactation, a reduction in the threshold for vasopressin secretion reduces osmolality, leading to retention of body water to aid lactation. However, the mechanisms that lead to altered vasopressin secretion are unclear.

We made electrophysiological recordings from SON magnocellular neurosecretory cells *in vivo* in anaesthetised female virgin and lactating rats. Oxytocin and vasopressin neurons were distinguished by excitation following intravenous cholecystokinin, which only occurs in oxytocin neurons. The TRPV antagonist ruthenium red was perfused through the SON via a microdialysis probe for 1 h. Ruthenium red reduced the firing rate of some vasopressin neurons, and had no effect on others, but overall caused a reduction in the firing rate of the population in both virgin rats ($n = 11$) and lactating rats ($n = 8$; main effect of reproductive status, $P = 0.81$, main effect of time, $P < 0.001$, two-way repeated measures ANOVA), despite osmolality being ~ 10 mosmol kg^{-1} lower in lactating rats compared to virgin rats ($P < 0.01$, Student's *t*-test). Ruthenium had no overall effect on oxytocin neuron activity in virgin rats ($n = 7$; one-way repeated measures ANOVA, $P = 0.51$).

These results suggest that TRPV channels on vasopressin neurons are activated at lower osmolality during lactation, which might lower the threshold for vasopressin secretion. Therefore, increased TRPV channel activation in vasopressin neurons might cause increased water retention in pregnancy and lactation.

M20: Deletion of protein tyrosine phosphatase 1B from forebrain neurons does not prevent the onset of diet-induced infertility in female mice

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Obesity impacts a number of hormonal functions, including reproduction. Leptin, an adipocyte-secreted hormone, acts on the hypothalamus to inhibit food intake and increase energy expenditure and most obese individuals develop hyperleptinemia and leptin resistance. Leptin signalling is regulated by multiple mechanisms, including tyrosine phosphorylation. Protein tyrosine phosphatase 1B (PTP1B) is an important negative regulator of leptin signalling through the dephosphorylation of JAK2. PTP1B is a ubiquitously-expressed enzyme and shows enriched expression in areas of high leptin receptor expression in the brain, including in the hypothalamus. Consistent with its role as a negative regulator of leptin signaling, PTP1B knockout in mice results in lean and leptin hypersensitive animals, but the effects on high calorie diet (HCD)-induced infertility have yet to be established. In this study, we used neuron-specific PTP1B knockout mice, expressed on a DBA/2J background strain prone to diet-induced infertility, to elucidate whether PTP1B mediates the effects of HCD on the onset of female infertility. HCD-fed PTP1B knockout mice were somewhat protected from diet-induced obesity (82% of HCD-fed control body weight after 11 weeks on the diet, but 142% of normal chow-fed controls; both $P<0.05$), but showed no improvement in glucose homeostasis compared to their HCD-fed wild-type littermates. Examination of the average number of litters, age at which the dams delivered their last litter, and circulating LH levels revealed no improvement in diet-induced infertility in HCD-fed PTP1B knockout mice, compared to HCD-fed controls. This study indicates that PTP1B knockout in female mice does not prevent the onset of diet-induced infertility, in spite of moderately preventing diet-induced obesity. In contrast, we recently showed that deletion of another leptin signalling inhibitor, SOCS3, rescued mice from diet-induced infertility for about 1.5 months. Therefore, while it is likely that multiple factors contribute to diet-induced infertility, PTP1B does not appear to be a major player.

M21: TRPV1 expression in the supraoptic nucleus of pregnant rats

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Pregnant females have increased water retention to sustain placental blood supply for the developing child. Vasopressin is a neuropeptide hormone that promotes water retention and is secreted from the posterior pituitary gland by magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei. Secretion of vasopressin is activated by mechanosensitive TRPV channels that are present on the membrane of magnocellular neurons. TRPV (1, 2 and 4) channels are activated by membrane shrinkage due to high plasma osmolality (hyperosmolality). In pregnancy, water retention causes hypo-osmolality but vasopressin levels are similar to, or higher than, those in non-pregnant females. Therefore, the osmotic threshold for vasopressin secretion is lowered in pregnancy. However, the mechanism that reduces the threshold is unknown. Here, we tested the hypothesis that increased TRPV expression by vasopressin neurons decreases the osmotic threshold for vasopressin secretion in pregnancy. Blood samples and brains were collected from six female virgin and six late-pregnant (gestation day 21) Sprague-Dawley rats. Plasma osmolality was 305.6 ± 2.1 mosmol/kg in virgin rats and 293.4 ± 2.9 mosmol/kg in late-pregnant rats ($P < 0.0001$, unpaired t-test), confirming reduced osmolality during pregnancy. Brains were sectioned through the supraoptic nucleus of the hypothalamus and double-label immunohistochemistry was performed for vasopressin and TRPV1. There was no difference in the percentage of double-labelled cells in the supraoptic nuclei of virgin (7.4 ± 1.5%) and pregnant rats (10.7 ± 2.8%, $P = 0.3115$, unpaired t-test). Therefore, a change in the numbers of TRPV1-expressing vasopressin neurons does not lower the osmotic threshold for vasopressin secretion in pregnant rats.

M22: Investigating the role of microglia in polycystic ovary syndrome (PCOS)

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Polycystic Ovary Syndrome (PCOS) is the leading cause of anovulatory infertility worldwide. Although typically thought of as an ovarian disorder, there is now clear evidence that the brain is involved. Changes in neuronal inputs to rostral preoptic area (rPOA) Gonadotrophin Releasing Hormone (GnRH) neurons have been identified in a prenatally androgenized (PNA) mouse model of the syndrome [1]. These changes to GnRH inputs are dependent upon androgen signalling and likely contribute to the downstream ovarian pathology. The mechanisms responsible for this modified neuronal circuitry within PCOS remain unclear. Microglia respond to inflammation and produce an extensive repertoire of neuroactive molecules that regulate synaptogenesis and axon migration [2]. We hypothesized that microglia may play a role in the modified brain wiring of PCOS. Microglial coverage and morphology were analyzed in different hypothalamic nuclei in PNA and control female mice (treated prenatally with dihydrotestosterone or a vehicle during gestational days 16-18) and treated with either flutamide (an androgen receptor blocker) or a VEH oil solution from postnatal day 40-60 (n=6-7/groups). Nickel-DAB immunohistochemistry with the Iba-1 primary antibody was used to stain for microglia and the microglial coverage and morphological state was analyzed with Image J software. Our initial results showed no treatment effect on microglial coverage within the rPOA or Arcuate Nucleus (ARN). However, our preliminary results of microglia morphology (representing activational state) within the ARN showed the number of microglia cells in the “surveying/resting” state (i.e. with thin ramified processes) is reduced in PNA mice following androgen receptor blockade. Taken together, our preliminary results suggest no change microglial coverage, but rather a reduction in the number of “surveying” microglia within the ARN following flutamide treatment within PNA mice. These preliminary results suggest that the neuronal plasticity in PCOS-like mice associated with androgen receptor blockade may involve changes in microglia activational state.

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M23: The role of RFRP neurons in murine puberty onset and anxiety

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RF-amide related-peptide (RFRP) neurons are thought to modulate reproductive function and stress responses[1]. Using transgenic mice which have stimulatory and inhibitory designer-receptors exclusively activated by designer-drugs (DREADDs) selectively expressed in RFRP neurons via a Cre-loxP system, we aim to explore the reproductive and behavioural effects of RFRP neurons non-invasively in vivo and to elucidate whether changes to RFRP neuronal tone can modulate puberty onset. To validate the efficacy of this model, we first investigated corticosterone concentrations from whole blood before and after stimulation of RFRP neurons using the synthetic DREADD ligand clozapine-n-oxide (CNO), or their inhibition with CNO during restraint-stress. RFRP neuronal stimulation was sufficient to elevate corticosterone levels from 36.1±7.9 and 46.1±5.9 ng/ml to 79.3±15.6 and 81.4±11.1 ng/ml, in females and males respectively ($p=0.036$ and $p=0.007$), whereas CNO was without effect in controls not expressing DREADDs ($p>0.05$). Inhibition of RFRP neuronal activity was insufficient to significantly abolish the corticosterone response to restraint-stress (restrained controls: 160.3±28.3 and 180.8±29.6 ng/ml vs restrained inhibitory DREADD-expressing mice: 201.4±31.0 and 113.4±25.0 ng/ml, for females and males respectively; $p=0.38$ and $p=0.08$). Current experiments are examining the role of RFRP neurons in reproductive function. In postnatal-day 26 mice, RFRP neurons will be chronically stimulated or inhibited (5 days of CNO delivered in drinking water) until the onset of puberty. We hypothesise RFRP stimulation will delay puberty onset in females, while it may accelerate puberty onset in males, since RFRP-3 administration modulates gonadotropin release in a sexually dimorphic manner (inhibiting the LH surge in female mice and stimulating LH secretion in male mice[2]). Tests assessing anxiety-like and depressive-like behaviours will determine the effects of transient changes in RFRP tone on stress responses. Elucidating the role of murine RFRP neurons is an important step towards understanding their role and therapeutic potential in human infertility and mental illness.

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M24: Effect of circadian rhythms on leptin and insulin sensitivity

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Metabolic processes are tightly regulated by the central circadian clock which resides in the suprachiasmatic nucleus of the hypothalamus. Food intake and metabolism are controlled by the hypothalamus, which orchestrates the integration of the hormones leptin and insulin. During obesity and other metabolic syndromes, this essential interaction is impaired, and leptin and insulin resistance develop. Striking links between circadian rhythms and metabolism have been reported, which encouraged us to explore whether circadian disruptions may impair leptin and insulin sensitivity in the hypothalamus. To alter the circadian rhythm in mice, the usual 12h light/12h dark cycle was shifted forward by 6h every 6 days (comparable to a 6 hour jetlag flying east) for 72 days. This treatment led to an increase in body weight compared with mice on a constant 12h light/12h dark cycle and minor changes in food intake, energy expenditure as well as an increase in basal blood glucose levels. Leptin sensitivity was assessed by the ability of the hormone to activate its transcription factor STAT3, while insulin sensitivity was assessed by its ability to activate the IRS/PI3K pathway, in the arcuate nucleus, a key integration site of both hormones. Interestingly, we discovered that leptin and insulin sensitivity were altered by circadian disruption within the ARC. These data strongly suggest that circadian disruption leads to metabolic and glucoregulatory impairments, associated with central dysregulation of leptin and insulin signalling. Modern phenomena such as social jetlag and shift work have a striking relationship to metabolic disease. Our results may provide mechanistic insights into those associations. Deciphering the role of arcuate GABA neurons in fertility regulation with chemogenetic tools in vivo

M25: Bond graph modelling of glucose uptake in small intestine using CellML

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Nutrients, electrolytes and water are absorbed into blood through the mucosa of the small intestine. The primary route of absorption is via enterocytes that are epithelial cells lining the lumen. The uptake, transport and metabolism of nutrients activate signalling pathways and feedback mechanisms that regulate effects over a range of time and length scales (e.g. expression of glucose transporter proteins, insulin secretion, appetite regulation, and growth). Mathematical modelling of nutrient uptake can improve understanding of the complex feedback mechanisms and how they are disrupted in disease. Therefore it can be helpful in study of the disease and find a treatment in a shorter time. We are developing models of signaling pathways which form part of a more complex nutrient transport model involving several organs of the body.

The model allows one to examine the variables that are very difficult to measure experimentally, such as the concentration of nutrient in the intestinal lumen or in the blood capillaries around the intestine, and then ultimately to look at how that links to hormone regulation, signaling pathways, etc. Here we present a validated computational model of glucose uptake in CellML in the simulation environment OpenCOR, which is platform independent and open source.

Our model is been built in a modular format, which means all of the transporters were modeled separately and then they all imported to a single file and combined together. This model is based on Bond graph concept to satisfy conservation laws and thermodynamic principles. Bond graphs are graphical representations of the dynamics of a physical system based on the topology of energy exchange between different components. We regenerated an existing model of glucose uptake in enterocyte with CellML. The model contained SGLT1, GLUT2, membrane diffusion, NHE3, AE1 and Na/K pump. The model then validated against published experimental data. So far, we have modified the model by adding more transporter and try to make it more realistic to simplify the understanding of glucose and other nutrients uptake mechanism in the small intestine.

M26: Myoregulin has no effect on contraction or relaxation of isolated rat hearts

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Cardiac function is dependent on synchronous excitation, contraction and relaxation of the ventricular myocardium. Microscopically, this is determined by fluctuations in the cytosolic calcium (Ca^{2+}) ion concentration and the ensuing cyclical activation of myofilaments, which macroscopically results in the generation of force and a cardiac output. One of the main determinants of the cytosolic Ca^{2+} concentration is the activity of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) which utilises ATP hydrolysis to re-sequester Ca^{2+} ions from the cytosol back into the intracellular Ca^{2+} store, the sarcoplasmic reticulum (SR). SERCA activity is endogenously regulated by phospholamban (PLB) and sarcolipin (SLN), both of which interact with SERCA to reduce its affinity for Ca^{2+} and, therefore, reduce Ca^{2+} uptake into the SR.

In 2015, a micropeptide encoded by a putative long non-coding RNA named myoregulin (MLN) was discovered and characterised as a PLB analogue and SERCA inhibitor in skeletal muscle. Furthermore, knockout of MLN was shown to improve skeletal muscle performance and improve SR Ca^{2+} uptake. Whether MLN performs a similar function in cardiac muscle, however, has yet to be determined. Therefore, by assessing the contractility of Langendorff-mounted rat hearts this study aimed to characterise the role of MLN as a modulator of SERCA function and cardiac muscle contraction and relaxation. Using various doses of MLN we have found that MLN has no significant effect on contraction or relaxation of the isolated heart, leading us to conclude that its role as a SERCA inhibitor is not applicable to cardiac muscle.

M27: Is hyperuricemia a regulator of the mTOR complex in pancreatic β-cells?

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Research into the impacts of high serum uric acid (SUA, hyperuricemia) on populations has shown a significant relationship between hyperuricemia and the development of diabetes[1]. To understand this relationship, we are investigating the impact of hyperuricemia on the regulation of the mechanistic target of rapamycin (mTOR) complexes in mouse and human pancreatic β-cells. These complexes are responsible for the regulation of cell growth, proliferation and survival while inhibiting autophagy. The mTOR complex 1 and 2 are regulated by DEP Domain Containing mTOR Interacting Protein (DEPTOR), which is the main focus of this investigation as it has been shown to inhibit both mTOR complexes under normal physiological conditions. In addition to this we will establish the 'cellular uric acid homeostasis' model in the pancreas by investigating endogenous expression of the uric acid transporters GLUT9, ABCG2 and MRP4 as well as xanthine oxidase (XO) and their change in expression in response to hyperuricemia.

Preliminary data have shown that all proposed uric acid transporters, mTOR, XO and DEPTOR are expressed in pancreatic cells measured by using real-time PCR and western blot analyses. Initial MTT assays measuring cellular metabolism have also shown a trend of decreasing cell viability under hyperuricemic conditions. This would suggest that hyperuricemia is affecting the viability of pancreatic beta cells. We are further determining the contribution of DEPTOR and the mTOR complexes to this observation providing a potential explanation of the loss of beta cell mass in diabetic patients with hyperuricemia.

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M28: Understanding the pro-apoptotic role of microRNA-532 in the diabetic heart

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Hyperglycaemia in diabetes promotes cell death among cardiomyocytes either by apoptosis and/or necrosis. Early molecular alterations have been shown to be accelerating this cell death process in response to the hyperglycemic conditions. MicroRNA-532 (miR-532), was originally demonstrated to have oncogenic properties. However, the recent studies show that miR-532 could regulate apoptotic cell death. Preliminary studies showed a significant increase in the expression of miR-532 in the human and mouse diabetic hearts as well as in HL-1 mouse atrial cardiomyocytes following long-term exposure to high glucose.

Therefore, the aim of this study is to investigate the effect of therapeutic inhibition of miR-532 in high glucose treated human cardiomyocytes and *ex-vivo* cultured human right atrial appendage (RAA) tissue

To determine if therapeutic modulation of miR-532 would influence cell death in human cardiomyocytes, the culture protocol for AC-16 human ventricular cardiomyocytes will be initially optimised. Once the culture protocol is optimised and the expression of miR-532 is confirmed, the activity of miR-532 will be inhibited using anti-miRs to assess its effects on the expression of potential target proteins, as well as the levels of apoptosis in the cardiomyocytes.

To translate the findings into the whole heart scenario, right atrial appendage tissue will be collected from patients undergoing coronary artery bypass graft surgery at the Dunedin hospital through Heart-Otago. Tissue will be cut into 1mm pieces and cultured in growth media supplemented with serum and transfected with anti-miR-532. Preliminary data indicate successful inhibition of miR-532 activity at both 48 and 72hours. Proteins will then be extracted from the tissues and the effect of the inhibition of miR-532 on cell survival will be measured by using caspase 3/7 assays on the proteins extracted.

Positive results from this study will not only identify a novel mechanism for increased apoptosis in diabetes, but also a therapeutic approach to improve the survival of diabetic cardiomyocytes.

M29: Soluble factors released by MDA-MB-231 breast cancer cells delay neutrophil apoptosis *in vitro*

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Recent research suggests that neutrophil lifespan and phenotype might be altered under certain physiological or pathological conditions including cancer. The objective of this study was to investigate whether soluble factors secreted by breast cancer cells (MCF-7 and MDA-MB-231) might alter the lifespan or phenotype of human neutrophils.

Freshly isolated human neutrophils were cultured in either control medium or conditioned medium (CM) produced by MCF-7 or MDA-MB-231 breast cancer cells. Neutrophil apoptosis was measured over a 48 hour period via flow cytometry using an Annexin V labelling/propidium iodide exclusion assay. Neutrophils incubated in control medium showed a significantly higher proportion of apoptosis compared to neutrophils cultured in MDA-MB-231 CM. No difference in apoptosis was observed between neutrophils cultured in control media compared to MCF-7 CM, suggesting that soluble factors secreted by MDA-MB-231 cells, but not by MCF-7 cells, were enhancing the lifespan of the neutrophils.

Quantitative RT-PCR was used to measure the relative expression of two anti-apoptosis proteins (A1 & MCL), three pro-apoptosis proteins (BAX, BAK, FAS) and one neutrophil activation marker (ICAM-1). Neutrophils cultured in control media or MCF-7 CM showed significantly lower expression of A1 and ICAM-1 and significantly higher expression of BAK compared to those incubated in MDA-MB-231 CM, consistent with a decrease in neutrophil apoptosis in MDA-MB-231 CM. No difference was noted in the expression of BAX, FAS or MCL when comparing neutrophils cultured in control medium, MCF-7 CM or MDA-MB-231 CM.

Lastly, western blotting of extracts from neutrophils cultured in control or MCF-7 CM showed extensive caspase-8 cleavage and activation associated with apoptosis, whilst those cultured in MDA-MB-231 CM showed little activation of caspase-8.

Taken together these results suggest that soluble factors secreted by MDA-MB-231 breast cancer cells enhance neutrophil survival by inhibiting apoptosis.

M30: The role of beta blockers in cardiac structural remodelling

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Type II diabetes is an epidemic in New Zealand affecting approximately 300,000 individuals. Patients with diabetes have an increased risk of developing cardiovascular disease. Heart failure is a form of cardiovascular disease characterised by structural changes to the heart. Such changes include cardiomyocyte apoptosis and fibrosis, which are increased in heart failure. Beta blockers are a common treatment for cardiovascular disease. This study aims to examine the efficacy of two beta blockers - metoprolol and carvedilol - in reducing cardiac structural remodelling during diabetic heart failure. Existing literature suggests that carvedilol may be a better alternative for diabetic patients.

16 week old male ZDF rats (n=30) were divided into 6 groups (n=5) based on diabetic status and treatment administered. Echocardiography was performed weekly and animals were sacrificed after 4 weeks of treatment. Stains for the relative measures of apoptosis and fibrosis were carried out on left ventricle tissue samples.

In contrast to the literature, which illustrates the added benefits of carvedilol over metoprolol, we found that treatment with carvedilol was not better than metoprolol in reducing fibrosis. We expect that results from apoptosis detection experiments and heart function analysis (echocardiography) will add to our findings.

Diabetes and related cardiovascular disease are growing issues for our population. Currently it is an accepted idea that carvedilol is emerging as a better treatment for diabetic heart failure than metoprolol. Although this is portrayed in the literature, our study challenges this idea and suggests that further investigation is required to determine whether carvedilol is a better alternative to metoprolol.

M31: Changes in expression of CaMKII isoforms in ApoE-/- mouse aorta

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Coronary artery disease (CAD) continues to be the leading cause of mortality in the world and a major source of disability, particularly for the aged population[1, 2]. The presence of vascular diseases such as atherosclerosis, is a predisposition to life threatening events such as acute myocardial infarctions and strokes[3].

Pathologies occurring in the vasculature such as atherosclerosis, stenosis, and hypertension are characterised by endothelial dysfunction and increased migration and proliferation of vascular smooth muscle cells (VSMC)[4]. Different isoforms of calcium/calmodulin dependent protein kinase 2 (CaMKII) have emerged to play a central role in the regulation of vascular homeostasis[5]. Determining which CaMKII isoforms are present, and their expression pattern during early development of atherosclerosis remains an active field of research, and holds the key to the starting point for many potential vascular pathological treatments.

13, 16 and 20 week old ApoE-/- mice had their abdominal aorta harvested and analysed for CaMKII protein and RNA expression. Preliminary results show a shift from in CaMKII isoform. As the age of the mice gets older, CaMKII-δ increases, whilst CaMKII-γ decreases.

Previous work in our lab has shown that the inhibition of CaMKII through the administration of KN-93 attenuates the development and severity of atherosclerosis in ApoE-/- mice. The results of this study show the changes in CaMKII isoform expression, and thereby the potential target for treatments.

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M32: Arrhythmic activity and reduced cardiomyocyte contractility in right ventricular hypertrophy

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Pulmonary artery hypertension is an underdiagnosed and understudied disease that if left untreated will progress to right ventricular hypertrophy and heart failure. The role of cardiomyocyte calcium (Ca^{2+}) homeostasis in the development of hypertrophy and the eventual progression to failure remains unclear, particularly in the right ventricle (RV).

The aim was to investigate cardiomyocyte Ca^{2+} handling in compensatory right ventricular hypertrophy.

Adult Wistar rats (300 g) were injected subcutaneously with either saline (Control), or 60 mg kg^{-1} monocrotaline (MCT, n=7) to induce pulmonary hypertension. After 28 days, rats were euthanized and the hearts removed. Trabeculae from the RV were mounted in a force transducer on the stage of an inverted microscope and field stimulated to contract (0.2Hz or 1Hz) at room temperature. Simultaneous measurements of force and intracellular Ca^{2+} (using fura-2 A/M) were carried out.

At 28 days post injection MCT hearts had significant RV hypertrophy, with no signs of failure. At both stimulation frequencies, MCT had non-significant differences in Ca^{2+} transients in comparison to Control. MCT animals however showed reduced active contractile force (MCT: 35.8±6.1 mN/mm^2 vs Control: 18.9±4.2 mN/mm^2, p=0.045) and reduced myofilament Ca^{2+} sensitivity. MCT trabeculae also displayed spontaneous Ca^{2+} release events and associated contractions during quiescence, which were seldom seen in Control trabeculae.

These results show the contractile response was compromised in MCT trabeculae prior to the onset of heart failure. Additionally, MCT trabeculae showed a predisposition to spontaneous Ca^{2+} release events, suggesting the presence of an arrhythmogenic substrate during compensatory RV hypertrophy. This animal model may therefore provide a means of studying the cellular mechanisms of cardiac arrhythmogenesis as well as pulmonary artery hypertension.

M33: Hypoxia inhibits voltage gated K^+-channels in pulmonary arterial smooth muscle cells by mitochondrial complex IV isoform 2 triggered release of reactive oxygen species

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Hypoxic Pulmonary Vasoconstriction (HPV) is essential to avoid life-threatening hypoxemia by matching perfusion to ventilation. Although the underlying molecular mechanisms are yet incompletely understood, mitochondrial-derived reactive oxygen species (ROS) have been suggested to act as essential mediators in HPV. Potential targets of ROS are voltage-gated K^+-channels (Kv-channels), whose inhibition depolarize pulmonary arterial smooth muscle cells (PASMCs), thereby triggering Ca^{2+}-influx and - consequently - initiating PASMC-contraction and HPV, respectively. However, the identity of the primary oxygen sensor as well as the link between mitochondrial ROS-release and inhibition of plasmalemmal Kv-channels remained unknown. Here, we investigated the role of the pulmonary specific isoform 2 of mitochondrial mitochondrial complex IV (Cox4i2) in HPV and hypoxic ROS-release as well as its downstream-effect on plasmalemmal Kv-currents and membrane potential in PASMCs.

Isolated ventilated and perfused lungs from *Cox4i2*$^{-/-}$ mice lacked acute HPV. Downstream signaling determined by patch clamp measurements showed decreased hypoxia-induced cellular membrane depolarization and oxygen-sensitive Kv-currents in *Cox4i2*$^{-/-}$ PASMCs compared to wildtype, which could be restored by exogenous application of ROS (H_2O_2). Consistently, PASMCs from *Cox4i2*$^{-/-}$ mice showed no hypoxia-induced increase of intracellular Ca^{2+}. Furthermore, H_2O_2 mimicked hypoxic depolarization in wildtype PASMCs under normoxic conditions, whereas pharmacological inhibition of mitochondrial superoxide-release by S3QEL2 blunted hypoxia-induced increase in membrane potential.

In conclusion, Cox4i2 is essential for acute oxygen sensing by triggering release of mitochondrial superoxide, which - after conversion to H_2O_2 - contributes to cellular membrane depolarization and initiation of HPV.

M34: Prolactin effects on kisspeptin fibre expression in the paraventricular and supraoptic nucleus of the mouse

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In New Zealand, approximately 7.4% of births are premature, which increases the risk of long-term health problems in the offspring. Labour starts with uterine contraction and so appropriate activation of the uterus during delivery ensures the normal timing of birth. The stimulus for uterine contraction is the posterior pituitary hormone, oxytocin. We have shown that kisspeptin excites the neurons that secrete oxytocin only in late pregnancy and that the kisspeptin fibre density around oxytocin neurons increases during pregnancy. Prolactin is another hormone that that is proposed to signal pregnancy to the maternal brain. Both kisspeptin neurons and oxytocin neurons express prolactin receptors. Hence, we aim to determine whether prolonged subcutaneous (SC) prolactin infusion increases kisspeptin fibre density in the hypothalamic paraventricular and supraoptic nuclei (where the cell bodies of oxytocin neurons are located) in virgin wild-type mice. Immunohistochemistry (IHC) will be carried out for kisspeptin and oxytocin to determine whether kisspeptin fibres innervate the paraventricular and supraoptic nuclei after prolonged infusion (through SC mini-osmotic pumps for seven days) of ovine prolactin or vehicle. IHC for the canonical intracellular messenger activated by prolactin receptor, phosphorylated signal transducer and activator of transcription factor 5 (pSTAT5), will also be carried out to determine whether pSTAT5 is present in key cell populations in the brain that respond to prolactin and have prolactin receptors, as a positive control. This study will help us to understand whether prolonged activation of prolactin receptors, as occurs in pregnant mice, can increase kisspeptin fibre density around oxytocin neurons in virgin mice.

M35: Investigating the role of epithelial sodium channel (ENaC) as a shear stress sensor in endothelial cells

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ENaC (epithelial sodium channel) is understood to have an important role in blood pressure regulation within the kidney [1]. However, recent studies have found ENaC expression in non-epithelial cells i.e. endothelial cells. ENaC within endothelial cells has been suggested to demonstrate as a shear sensor [2]. Within the vasculature, ENaC is exposed to shear stress derived from the blood flow [3]. ENaC has been suggested to increase in sodium influx as a response to shear stress, resulting in endothelial dysfunction [4]. One of the key risk factors for the onset of cardiovascular diseases is endothelial dysfunction, which often results in impairment to the vascular tone or increased binding of inflammatory mediators, exacerbating atherosclerosis [4].

This present study aims to investigate the role and establish a model of ENaC as a shear stress sensor within endothelial cells. It is hypothesized that ENaC expression is dependent on shear stress. To address this study aim, endothelial cells will be cultured under different shear stress conditions, using a cell perfusion system. The expression levels of ENaC subunits (alpha, beta, gamma and delta) will be analysed under different shear conditions. ENaC will be analysed at messenger RNA (mRNA) level using qPCR and protein level using Western Blot techniques. With these findings, we aim to understand ENaC's role as a shear sensor in endothelial cells and how this role affects endothelial dysfunction.

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M36: Optimisation of hanging drop technique for in vitro differentiation of mouse embryonic stem cells into cardiomyocytes

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Myocardial infarction (MI) results in the loss of cardiomyocytes and decline in cardiac function. Despite the advances in the management of MI, current treatments do not address the loss of myocytes. Therefore, MI remains the leading cause of mortality worldwide. Recently, stem cell therapy is gaining momentum due to their ability to regenerate the cardiomyocytes, therefore, restoring the cardiac function. Among stem cells, pluripotent stem cells (PSCs) are being considered to be the therapeutically feasible stem cells due to their ability to proliferate indefinitely and differentiate into cardiomyocytes.

Given the background, it is important to understand the PSCs differentiation process into cardiomyocytes. Therefore, the current study aims to 1) optimise hanging drop technique for in vitro differentiation of mouse embryonic stem cells (mES) a model of PSCs into cardiomyocytes and 2) differentiate the mES into cardiomyocytes through inhibition of Wnt signalling pathway.

To optimise the hanging drop technique, cell numbers in a single droplet were tested with 25 cells, 100 cells, 500 cells, 1000 cells, 2000 cells, 2500 cells and 4000 cells per µl. Cell number of 100 cells/µl showed clear and higher probability of forming cell aggregates and embryoid bodies (EBs) compared to the other cell suspensions.

The hanging drops were incubated for two days for the EB formation. After 2 days, the EBs were treated with the Wnt signalling inhibitor XAV939 (1µM). Previous studies have shown that inhibition of Wnt directs the EBs to cardiomyocytes. Differentiation of EBs to cardiomyocytes was confirmed by immunocytochemistry which showed expression of cardiac specific proteins connexin 43 (Cnx43) and insulin gene enhancer protein (ISL-1) in differentiated cells.

In conclusion, we have optimised the ideal cell number (100 cells/µl) required for successful formation of EBs by hanging drop technique. In addition, inhibition of Wnt signalling leads to EBs differentiation into cardiomyocytes.

M37: Synergistic paracrine effects of cardiac progenitor cells from the right atrium and left ventricle on *in vitro* cultured cardiovascular cells

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Ischaemic heart disease (IHD) can result in the loss of cardiac tissue, decreasing cardiac function which can progress towards heart failure. Improvements in pharmacological and surgical interventions have led to better management of IHD but does not treat the underlying pathology of lost cardiac tissue. Stem cell therapy shows promising results on regenerating cardiac tissue. Cardiac progenitor cells (CPC) seem to be an ideal candidate for cell therapy and have been shown to be superior to other adult stem cell populations. Recently, we showed that CPCs isolated from different chambers of the heart exhibit functional differences *in vitro*. CPCs isolated from the right atrial appendage (RAA) have superior effects on cell survival while CPCs isolated from the left ventricle (LV) have superior effects on angiogenesis. As IHD is a combination of loss of cells and poor angiogenesis, this study aims to understand the synergy of RAA-CPCs and LV-CPCs on *in vitro* cultured cardiovascular cells.

CPCs were isolated from the RAA and LV of patients undergoing on-pump coronary artery bypass grafting (CABG). CPCs were characterised for mesenchymal cell markers, CD90 and CD105, and the circulating haematopoietic cell marker, CD34, using flow cytometry. Conditioned media (CM) was collected after exposing RAA-CPCs and LV-CPCs grown in serum-free conditions and cultured alone or in combination under normoxic and hypoxic conditions. The CM will be used to measure the release of growth factors, insulin growth factor 1 and vascular endothelial growth factor A, using enzyme-linked immunosorbent assay. The cell survival effects of CM will be measured by performing a caspase 3/7 assay on AC16 cardiomyocytes and the angiogenic effects of CM will be measured by performing a tube formation assay using human umbilical vein endothelial cells. Together, these results can demonstrate the synergistic paracrine effects of RAA-CPCs and LV-CPCs on *in vitro* cardiac repair.

M38: Identification of transporters involved in drug-drug interaction during gout treatment in primary rat hepatocytes

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Gout is the most common form of inflammatory arthritis and is mainly caused by chronically elevated serum uric acid (SUA) levels, or hyperuricemia. Allopurinol is the gold standard therapy for gout, with its urate-lowering effect mainly attributed to its active metabolite, oxypurinol. Hypertension is a common comorbidity of gout and is often treated with diuretics such as furosemide. However, concomitant treatment with furosemide compromises the therapeutic effects of allopurinol. The molecular mechanism underlying this adverse drug-drug interaction is not fully understood. Evidence from clinical studies suggests that complex interactions might be occurring in the liver, where allopurinol and oxypurinol act to lower SUA. Based on current knowledge of the transport of furosemide and allopurinol/oxypurinol by urate transporters in the kidney, we hypothesized that similar transporters exist in the hepatocyte of the liver, and might possibly be where furosemide exhibits its inhibitory effect on allopurinol. Urate transporters with affinities for the above drugs and found to be expressed in the liver were chosen as candidates. These are OAT2, OAT3, MRP4, GLUT9, NPT1, NPT4, and ABCG2. To investigate their presence in the rat liver, RT-qPCR and immunoblotting were performed to confirm gene and protein expression, respectively. Using primary rat hepatocytes as a functional model, transport of these drugs and their effects on each other will be examined with intracellular and extracellular uric acid measurements as functional outputs. Additionally, the contribution of candidate transporters to the transport of allopurinol and/or oxypurinol will be investigated by application of specific transporter inhibitors and siRNAs.

M39: Understanding altered heart rate generation in type 2 diabetes

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Type 2 diabetic patients present an incompetence in heart rate regulation increasing their risk of life threatening arrhythmias. We recently found *in vivo* heart rate was comparable between type 2 diabetic rats and non-diabetic controls; however, intrinsic heart rate (i.e. rate without neuronal control) was decreased in diabetic animals. Why the intrinsic heart rate is different remains unknown.

Heart rate originates in the sinoatrial nodal cardiomyocytes located in the right atria, and is initiated by rhythmic oscillations in Ca^{2+} and other ions. Transient changes in these ions give rise to what are termed the Ca^{2+} and membrane clocks which combined set the heart rate. The Ca^{2+} clock primarily involves the intracellular Ca^{2+} store, the sarcoplasmic reticulum, and is mediated by a collection of 'Ca^{2+}-handling' proteins. The membrane clock involves various cell membrane ion transporters, enabling respective ion flux across the cell membrane. Heart rate is propagated to the rest of the heart via proteins known as connexins.

The **aim** of this study was to investigate whether a reduced intrinsic heart rate in diabetes is due to changes in the Ca^{2+} and/ or membrane clocks.

To this end, right atrial tissue has been isolated, fixed and sectioned. Martius scarlet blue histology stain was used to locate the sinoatrial node. Sinoatrial nodal cells stain purple and are embedded in blue connective tissue. Confirmation of sinoatrial node identification was performed using immunofluorescence. This was achieved by labelling a nodal specific ion channel as a positive marker and observing the lack of connexin 43 as a negative marker. To date, all Ca^{2+} clock and membrane clock proteins have been optimised for immuno-fluorescence but no comparisons between diabetic and non-diabetic rats have been made. Presently *in vivo* and intrinsic heart rate recordings are being taken in diabetic and non-diabetic rats prior to atrial tissue isolation.

M40: Finding CLARITY: Visualising the gonadotrophin-releasing hormone neuron

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The Gonadotrophin-Releasing Hormone (GnRH) neuron is the final downstream neuron of the neuronal network controlling reproductive function. The GnRH neurons are dispersed throughout the rostral forebrain in a diffuse distribution, possessing remarkably long dendritic processes. This makes targeting and visualising the neurons for anatomical studies using classical approaches particularly challenging. Previous studies suggest long dendrites of GnRH neurons project millimetres through the rostral forebrain towards the median eminence (ME), and that some dendrites then transition into axons that ramify throughout the ME. However it has not been possible to visualise the entire dendritic projection of individual neurons located in the rostral preoptic area (rPOA). This study used advances in viral-mediated transduction tools combined with optical clearing to assess GnRH neuron morphology in its entirety. Female GnRH-cre mice (n=4) were injected with cre-dependent AAV channel rhodopsin (ChR) linked to mCherry, into the rPOA, to drive the expression of ChR-mCherry, which becomes docked in the membrane exclusively in GnRH neurons. Thick (1.5 mm) brain slices were cleared using the CLARITY method, immunostained for mCherry, and imaged using a Nikon 1A confocal with long-working distance objective. mCherry expression was restricted to GnRH neurons in the rPOA and thin fibre processes. Dendritic projections were indentified extending all the way to the ME and subsequently branching. Morphological features of the GnRH neuron, such as spines, could also be visualised along the length of the dendrite. Combining the viral delivery of conditionally expressed fluorophores into a select population of GnRH neurons, coupled with optical clearing is currently a promising approach to image the entire GnRH neuron *in situ* without risk of tissue loss. These findings support the presence of a dendrite-axon process in rPOA GnRH neurons, and raise additional questions about the functional role of this unique structure in the regulation of fertility.

M41: Long-term effects of exercise on zinc homeostasis

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Long-term impact of exercise on zinc status is unclear. We conducted a systematic review of articles published up to 28th January 2016 to identify studies that investigated the zinc status of trained populations, compared to untrained controls, using one or more zinc biomarkers. Six interventional studies (n=89 exercise, 86 controls) and 16 cross-sectional studies (n=1374 athletes, 670 controls) were eligible for inclusion. In cross-sectional studies, the majority of athletes displayed lower serum zinc concentrations and higher dietary zinc intakes, compared to controls. Interventional studies provided inconclusive results on differences in zinc biomarkers between exercise and control groups. Further investigations are required to determine zinc requirements for athletes and exercise-induced changes in zinc homeostasis. The limited evidence suggests that those who regularly exercise have lower serum zinc levels, which appear to be independent of dietary zinc intake.

M42: Downregulation of non-neuronal cholinergic system (NNCS) reduces glucose transporter in the diabetic heart

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A non-neuronal cholinergic system (NNCS) comprises of acetylcholine (ACh)-synthesizing enzyme, transporters, degrading enzyme and receptor. Recent studies have shown that activation of NNCS enhances cardiac glucose uptake by upregulating the glucose transporter-4 (Glut-4) expression and translocation, allowing the heart to adapt to the ischemic condition. Since reduced glucose transport predisposes the diabetic heart to extensive ischemic damage in the event of myocardial infarction, modulation of NNCS could be a therapeutic option. However, the involvement of NNCS in the diabetic heart is currently unknown. Therefore, this study aims to determine the role of NNCS, specifically the ACh synthesis (by choline acetyltransferase, ChAT) and type-2 muscarinic receptor (M_2AChR) which involve in NNCS activation, in relation to glucose uptake (Glut-4) in the diabetic heart.

Heart tissues derived from (i) Type-2 diabetic and age-matched non-diabetic mice (8- to 20-weeks of age) and (ii) Type-2 human diabetic and non-diabetic heart with ischemic heart disease were used in this study. Western blotting was performed to measure the protein expression of ChAT, M_2AChR, and Glut-4. In the mouse diabetic heart, the ChAT expression was significantly reduced at 8-weeks of age, indicating reduced ACh synthesis at an early stage of diabetes. M_2AChR expression was significantly reduced in 8-, 16- and 20-weeks old diabetic mice, indicating reduced NNCS activation. Glut-4 expression was significantly increased in the 8-weeks old diabetic mice in response to hyperinsulinemia. However, it was significantly decreased in 20-weeks old diabetic mice, indicating reduced glucose uptake. In the type-2 human diabetic heart, a significant reduction was observed in both Glut-4 and ChAT expression while M_2AChR expression remained unchanged. In conclusion, these preliminary results suggest that downregulation of NNCS may decrease glucose uptake through Glut-4 expression in diabetic mice and human, warranting further investigation.

M43: Does hyperuricemia control ubiquitination of p53 via OGT in cancer?

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Humans are predisposed to the development of hyperuricemia, defined as a plasma uric acid concentration greater than 360µmol. Hyperuricemia has been associated with multiple pathologies including gout, diabetes, metabolic syndrome and cancer. Although the consequences of hyperuricemia have been defined in prostate cancer, a model and mechanism of uric acid transport in breast cancer has not yet been established. An overt and aberrant display of the fundamental concept of energy acquisition can be observed in cancer cells. This is often accompanied with hypersensitivity to energy depletion in which limiting conditions induce O-GlcNAcylation/O-linked N-AcetylGlucosamine Transferase (OGT) dependent apoptosis and proteasome dependent degradation. The aim of this study is to determine if hyperuricemia controls ubiquitination of p53 via OGT/O-GlcNAcylation in breast cancer.

We have cultured low aggressive breast cancer cell line MCF7 in Williams E Media (WEM) and HEK media with various supplements. Preliminary experiments have confirmed expression of uric acid influx and efflux transporters; GLUT9, MRP4 and ABCG2 in WEM cultured MCF7 cells at the RNA level via RT-qPCR. RNA presence of Xanthine Oxidase (XO) and the lack of insulin dependence of GLUT9 expression in MCF7 cells has also been analysed using this method. RNA expression data have all shown to be statistically significant. Expression of OGT, XO and p53 have been confirmed at a protein level via immunoblotting. Treatment of MCF7 cells with increasing concentrations of uric acid have shown to increase proliferation according to MTT proliferation assays. We further aim to determine if hyperuricemia changes OGT expression and the consequences for p53 stability.

M44: Is treating the transient post-stroke hypertension after ischemic stroke beneficial? A Wistar rat model study

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Over 80% of patients show a transient post-stroke hypertension, however whether the increased pressure is necessary to enhance tissue perfusion remains uncertain. We aimed to determine whether controlling blood pressure (BP) in a rat model of ischemic stroke, using the clinically-indicated calcium channel blocker, nifedipine, is beneficial.

Male Wistar rats (411±10 g) were instrumented to allow long-term recording of BP, intracranial pressure (ICP) and brain tissue oxygen (pO_2) via telemetry. After a recovery period, baseline data was recorded, and on Day 0 an ischemic stroke was induced, via a two hour occlusion of the middle cerebral artery, in Untreated (n= 6) and Treated groups (nifedipine 1.5mg/kg/hr sc, n=8). Behavioural testing was performed on Days 1, 3, 7 and 10 to evaluate the functional recovery.

After stroke, BP increased rapidly in Untreated rats, reaching a peak of 37±4mmHg above baseline at 12±6 hours vs 14±5 mmHg in Treated ($p<0.05$). Cerebral perfusion was significantly elevated on Day 0 in Untreated, and Days 0 and 1 in Treated rats. The increase in pO_2 observed in penumbra for several days after stroke was not significantly different between the treated and untreated rats. Post-stroke functional recovery was also not significant between groups, despite the infarct volume appearing larger in Treated animals (248±23mm^3 vs Untreated 147±28mm^3; $p<0.05$).

Treating post-stroke hypertension does not appear to have a major impact on functional recovery in young, normotensive rats. Future studies will examine the impact of factors such as chronic high blood pressure or old age.

M45: CaMKII inhibition reduces foam cell lesion development in the brachiocephalic artery of apolipoprotein $E^{-/-}$ mice

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Atherosclerosis is the major cardiovascular disease characterised by the formation of lipid-laden lesions within the inner layer of the arterial wall. Current therapies target at lowering LDL-cholesterol (LDL-C), however, even when the LDL-C target is met, there remains a residual risk for a cardiovascular event. This suggests additional therapies targeting other pathways need to be developed.

Reactive oxygen species (ROS) are highly damaging molecules that promote inflammation. It is now well recognised that sub-acute, chronic inflammation drives many of the pathogenic mechanisms underlying atherosclerotic lesion development. Calcium calmodulin-dependent protein kinase II (CaMKII) phosphorylates a number of cellular proteins, in particular, those involved in Ca^{2+} and ROS signalling such as endothelial nitric oxide synthase (eNOS). Under physiological conditions CaMKII is only briefly active through an autophosphorylation/dephosphorylation mechanism driven by calcium events. However, under conditions of elevated oxidative stress CaMKII becomes oxidised, rendering it persistently active. The role chronically active CaMKII plays throughout the progression of atherosclerosis is yet to be established.

Apolipoprotein E knockout ($ApoE^{-/-}$) mice were administered the CaMKII inhibitor KN-93 or control compound KN-92 every second day from 16- to 20-weeks of age. The brachiocephalic artery was dissected and a serial set of sections obtained for plaque analysis and immunohistochemistry. Systemic CaMKII inhibition led to a 10.9% reduction in foam cell lesion size in the brachiocephalic artery at 20-weeks ($P<0.05$). Furthermore, the reduced lesion size was associated with a significant decrease in phosphorylated and oxidised CaMKII, as well as the superoxide producing phosphorylated eNOS (P^{1177}-eNOS).

This study is the first to identify chronically active forms of CaMKII within atherosclerotic lesions. Collectively, these findings have implicated a potential mechanism contributing to atherosclerosis and spotlight post-translational modifications of CaMKII as a novel target for controlling the progression of early foam cell lesion development.

M46: Ventricle specific cardiomyocyte differentiation of mouse embryonic stem cells

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Acute myocardial infarction leads to loss of large number of cardiomyocytes causing severe ventricular tissue damage. Pluripotent stem cells (PSC) are gaining popularity as the ideal source to regenerate the lost cardiomyocytes, due to their capability to proliferate and differentiate into cardiac lineage. Various differentiation protocols have been established to differentiate PSC into cardiomyocytes, however all resulted in the development of a heterogeneous (atrial, nodal and ventricular) population of cardiomyocytes. Unfortunately, once transplanted to the left ventricle, these nodal cells will generate unwanted ventricular arrhythmias. We aimed to design a protocol to differentiate mouse embryonic stem cells (mESC) into ventricle-specific cardiomyocytes. By inhibiting Wnt signaling in the early stage, we aimed to trigger mESC to develop into cardiomyocyte lineage while inhibition of Retinoic acid signaling in the later stages would trigger a ventricular phenotype. To enhance the differentiation, ascorbic acid was added throughout the process. Gene expression of stage specific cardiomyocyte markers was validated by qPCR and protein expression was validated by flow cytometry and immunofluorescence analysis after 14 days of differentiation. Our preliminary results showed that all the major cardiac markers (*Isl-1, Nkx2.5, Mef2C, CTnT, IRX4,* and *MLC2V*) were expressed in a stage specific manner. Importantly, ~75% of cells were expressing the ventricle specific IRX4 after 14 days of differentiation (n=3). Our approach is the first step to the development of a differentiation protocol for ventricle-specific cardiomyocytes, which will have a significant contribution to the field of cardiac regeneration therapy.

M47: Differential regulation of cerebral and renal blood flow by the carotid body in health and hypertension

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Hypertension affects 40% of adults and is the leading cause of disability-adjusted life-years lost. Recent studies show that aberrant afferent signalling originating from the carotid bodies (CBs) contributes to chronic increases in sympathetic nerve activity, total vascular resistance, and mean arterial pressure (MAP). Given the location of the CBs at the bifurcation of the common carotid arteries, it has been proposed that CB-mediated increases in MAP occur in order to maintain cerebral perfusion; however, the effect of CB activation on cerebral perfusion remains ambiguous. We therefore determined the cerebral haemodynamic response to CB activation in the conscious, normotensive and hypertensive (two-kidney, one-clip) sheep, and compared this to the response in the kidney.

MAP, blood flow, and vascular conductance in the common carotid and renal arteries were measured at rest and in response to CB stimulation using intracarotid potassium cyanide (KCN; 20 μg/kg) during normotension (baseline MAP = 74.3±4.1 mmHg; baseline carotid blood flow = 643.8±44.5 mL/min) and 10 days following renal artery clipping when sheep were hypertensive (baseline MAP = 96.5±9.5 mmHg; baseline carotid blood flow = 560.4±50.8 mL/min). During normotension, CB stimulation caused increased MAP, carotid blood flow (27.0±6.2%), and carotid vascular conductance (23.2±5.4%), whilst renal blood flow remained unaltered despite decreased renal vascular conductance (-12.5±3.2%). Following parasympathetic blockade with intravenous atropine (8 mg bolus followed by 0.8 mg/min for 30 minutes), the carotid blood flow (36.7±3.6%) and carotid vascular conductance (32.5±6.7%) responses to KCN were augmented. Increased carotid blood flow (33.9±3.7%) and carotid vascular conductance (30.4±2.7%) responses were also observed during hypertension.

These preliminary results suggest that CB activation causes increased cerebral blood flow that is partly driven by vasodilation of the cerebral vasculature. In contrast, CB activation causes renal vasoconstriction with preserved renal perfusion due to the concomitant rise in MAP. Overall, the CB appears to play a protective role in maintaining perfusion to the brain in both normotensive and hypertensive states.

M48: Aortic chemoreceptor stimulation increases coronary blood flow in the conscious sheep

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Peripheral chemoreceptors (aortic and carotid bodies) are putative chemical sensors that detect and respond to changes in arterial oxygen. Reflex responses by carotid bodies to acute hypoxia have been well characterised but the exact role of aortic bodies is unclear. We hypothesized that selective aortic body stimulation using potassium cyanide (KCN 10/20/30μg/kg) into the left ventricle would increase coronary blood flow (CoBF) and coronary vascular conductance, with these responses attenuated after parasympathetic-blockade by atropine (32mg/30min).

Adult sheep were instrumented with transonic flow probes around the left circumflex artery and aorta, and diaphragmatic electromyography electrodes under general anaesthesia. In the conscious resting state, aortic body stimulation resulted in a dose-dependent increase in CoBF (123 ± 17% over baseline; n = 4 for KCN 30μg/kg) and was associated with increased blood pressure and breathing frequency. Coronary vascular conductance was also elevated (107 ± 15% over baseline; n = 3 for KCN 30μg/kg) despite the increase in blood pressure. Both CoBF and conductance responses were attenuated by atropine (61 ± 14%; n = 4 , 36 ± 23%; n = 3, respectively). Our novel results suggest that aortic bodies respond to hypoxia by increasing coronary blood flow at least in part, via parasympathetic nerve-mediated coronary vasodilatation. This, together with their anatomical location immediately upstream to the coronary vasculature suggests a specialised role of aortic bodies in maintaining adequate oxygen supply to the heart.

M49: Stretchable sensors based on CNTs/PDMS composites capable of detecting tension and pressure

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With people paying much more attention to health, there is a dramatic increase in the healthcare costs. In particular, wearable sensing devices, which can monitor inhabitants without hospitalization, have gained growing attention[1-3]. There are several requirements for wearable sensors to ensure that it is possible to put them into practical application. For wearable sensing devices, the stretchability and sensitivity are the main requirements[4-6]. Polymer/CNTs composite is a promising candidate to serve as sensing material, due to the high elasticity of the polymers and excellent electrical properties of CNTs as well as benign compatibility with polymers. Apart from these, the versatility of sensors is usually taken into consideration, such as two or more force detection. So if the wearable sensing device can obtain these properties, it is promising to be employed widely in monitoring devices for humans[7].

Herein, a novel sensing device is proposed with desirable characters and versatility. The sensing device is designed to sense the strain through the variation of conductive areas, with different structure in longitude and transverse direction generating different resistance responses. There are three layers of the stretchable sensors, the top is a layer of stretchable conductive electrode, the CNTs/PDMS with serpentine-prism structure in the middle and the bottom layer is PDMS as substrate. In the initial phase, the sensing section CNTs/PDMS composite film was fabricated using a facile coating method. The PDMS/CNTs composite film not only exhibited an excellent stretchability that can be stretched as high as 100% as its original length, but also shew strong piezoresistivity with good and tunable gauge factors. Next, the stretchable conductive electrode is prepared and characterized, which then is put on the layer of CNTs/PDMS composite with serpentine-prism structure. The combined sensor is characterized regarding detecting pressure and tension, maintaining the stretchability and sensitivity. Meanwhile, a simulation study will be conducted along with the whole experiment by comparing the real results with forecasting results. This research may offer not only a multifunctional sensing device but also the sensing device is promising to be employed in wide healthcare applications.

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Medsci Plenary Lecture #2: Growth hormone in sports: Detecting the doped or duped

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Growth hormone (GH) has been abused in sports for decades and at least 10 years before its availability to treat adults with GH deficiency. While believed to be widely abused by athletes, there is limited evidence that GH improves physical performance in healthy adults or in trained athletes. A large placebo-controlled trial in recreational athletes has reported that GH enhances sprint capacity but not muscle strength, power, or aerobic exercise capacity an effect enhanced by anabolic steroids.

Doping with GH is difficult to detect because the hormone is produced endogenously. There are two approaches both approved by the World anti-Doping Agency. The first known as the Isoform method was introduced in 2004. This relies on the detection of recombinant monomeric 22 KD isoform and distinguishing it from the endogenous pituitary product, which is made up a mixture of GH isoforms that is suppressed by exogenous administration. The second approach, known as the Biomarker method was introduced in 2013 during the London Olympics. It is based on the detection of elevated concentrations of GH responsive proteins, specifically IGF-I and procollagen III, which are the best characterised and validated. Because of the short half-life of GH, the isoform Method is used principally in out-of-competition testing. Both methods have successfully detected GH abuse that has led to successful prosecution and suspension. Novel approaches that include gene expression and proteomic profiling are being investigated to expand the repertoire for detection.

S1A.1: Impaired microcirculation and insulin resistance

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Skeletal muscle is an important site for insulin-mediated glucose disposal in the post-prandial period. The classical action of insulin to increase skeletal muscle glucose uptake involves insulin binding to insulin receptors on myocytes to stimulate glucose transporter 4 translocation to the cell surface membrane, thereby enhancing glucose uptake. However, an additional role of insulin on muscle glucose metabolism is its role to increase muscle microvascular perfusion thereby facilitating insulin and glucose delivery to the myocyte. This microvascular action of insulin accounts for 40-50% of insulin-stimulated glucose disposal in skeletal muscle. Myocyte and microvascular responses to insulin may be impaired with insulin resistance resulting in decreased glucose disposal by skeletal muscle. Growing evidence suggests that the loss of microvascular insulin action in skeletal muscle has important physiological consequences in the early pathogenesis of insulin resistance.

S1A.2: The role of vascular ENaC in impaired flow-mediated responses of carotid arteries in diabetes

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Type 2 diabetes contributes to many cardiovascular complications, associated with poor blood flow relating to impaired vessel flow-mediated dilation. We have recently demonstrated that flow-mediated arterial responses are dampened by activation of the epithelial sodium channel (ENaC) expressed in the endothelium. Hyperglycaemia is known to increase ENaC activity and expression in the kidney. Therefore, we hypothesised that increased endothelial ENaC activity contributes to impaired flow-mediated responses in diabetes.

Twenty week old male Zucker Diabetic Fatty rats (DM) exhibited elevated blood glucose compared to their non-diabetic littermates (nDM) (30.7 ± 0.7 compared to 11.3 ± 0.6 mmol/L; $p < 0.0001$). Isolated carotid arteries were mounted at 60 mmHg in a pressure myograph, and changes in outer diameter (OD) assessed.

Endothelial dysfunction was observed in DM; with a reduced vasodilator response to 10µM acetylcholine (+22 ± 9 compared to +44 ± 04 µm, $p = 0.0004$), despite similar contraction to 10µM noradrenaline. Blockade of ENaC (10µM amiloride) resulted in significant dilation, similar in both nDM (+128 ± 18 µm) and DM (+128 ± 23 µm). Stepwise increases in intraluminal flow, without alteration of mean intraluminal pressure, decreased OD, with maximal reductions of -56 ± 30 µm and -81 ± 21 µm in nDM and DM, respectively. Interestingly, this constrictive response to flow was significantly reduced when ENaC was blocked in nDM (-21 ± 25 µm), but not in DM (-67 ± 43 µm).

Our results demonstrate a functional role for vascular ENaC in rat carotid arteries under normal conditions; contributing to spontaneous tone and dampening flow-mediated responses. Remarkably, DM did not show significant alteration of isolated vascular function, suggesting factors external to the arteries per-se are responsible for the well-known impairment of flow-mediated responses. Contrary to our original hypothesis, our data point to a reduction in ENaC-sensitive flow-mediated response in DM.

S1A.3: Endothelial glycocalyx – critical roles in vascular dysfunction in diabetes

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Aims: Widespread vascular dysfunction accompanies albuminuria in diabetic and non-diabetic nephropathies. This vascular dysfunction includes accelerated atherosclerosis, impaired vasodilatation, decreased nitric oxide bioavailability, and increased permeability. We aim to determine whether endothelial glycocalyx disruption underlies widespread vascular dysfunction in diabetes and other kidney diseases.

Background: Endothelial glycocalyx is a gel-like matrix covering the luminal surface of all blood vessels. Experimental disruption of endothelial glycoclayx causes the same pattern of abnormalities as seen in nephropathy (altherosclerosis, impaired vasodilatation, decreased NO, increased permeability). Restoring the endothelial glycocalyx is feasible, and improves the function of damaged capillaries (Salmon, Cardiovasc Res, 2009).

Methods: I have used single capillary permeability techniques in vivo to measure capillary wall permeability coefficients directly, in structurally distinct capillary beds (fenestrated capillary: glomeruli, and continuous capillary: gut mesentery), in adult male Sprague-Dawley rats 7 days after streptozotocin (45 mg/kg iv). Animals were anaesthetised, the gut mesentery exteriorised, single microvessels cannulated and perfused, and hydraulic conductivity (L_P) calculated. Single glomeruli were subsequently harvested from the same animals, and volume-corrected ultrafiltration coefficient (L_PA/V_i) measured using an oncometric technique (Salmon, J Physiol, 2006).

Results: Mesenteric L_P and glomerular L_PA/V_i were both increased in proteinuric diabetic animals (both $p<0.05$, unpaired t-test). In non-diabetic nephropathy (Munich-Wistar-Fromter rats), we have also demonstrated identical widespread alterations in permeability of mesenteric and glomerular microvessel (both $p<0.05$, unpaired t-test), and novel confocal/multiphoton microscopy techniques revealed accompanying endothelial glycocalyx disruption in mesenteric and glomerular capillaries.

Conclusion: Widespread alterations in permeability occur early in these diverse forms of kidney disease, and coupling single vessel permeability and confocal/multiphoton microscopy studies identifies altered endothelial glycocalyx as a potential link between albuminuria and widespread vascular dysfunction.

S1A.4: Early downregulation of proangiogenic microRNAs leads to microangiopathy in diabetic heart

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Diabetes induced microangiopathy as a result of endothelial dysfunction is a major contributing factor for the development of heart disease in individuals with diabetes. Dysregulation of endothelial-specific microRNAs (miRs) is correlated with impaired angiogenesis and cell survival. We aimed to determine the role of two proangiogenic miRs, miR-126, and miR-132 in diabetes induced microangiopathy.

The presence of diabetes alone significantly decreased the expression level of both miR-126 and miR-132 in the plasma and the myocardium collected from patients undergoing coronary artery bypass graft surgery. The downregulation of both miRs was also associated with reduced number of capillaries and arterioles and increased endothelial cell apoptosis, the hallmark of microangiopathy. A time course study using a mouse model of type-2 diabetes confirmed that the downregulation of miR-126 and miR-132 preceded endothelial apoptosis as well as alterations in the density of the microvasculature. Interestingly, overexpression of both miR-126 and miR-132 in diabetic aortic rings abrogated the deleterious effects of diabetes on cell survival and proliferation and restored their angiogenic potential.

These novel findings, therefore, demonstrate that early downregulation of proganiogenic miRs is a major mechanism underlying the development of microangiopathy in diabetic hearts suggesting that therapeutic restoration of proangiogenic miRs could be a potential approach to combat the cardiovascular complications of diabetes.

S1B.1: The effect of general anaesthesia on sleep and the circadian clock

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The overarching goal of our research is to understand how general anaesthesia (GA) affects the circadian clock and sleep post-operatively using animal models and to develop ways to combat post-operative sleep and circadian disruption in patients using the principles of chronobiology. In this talk I will discuss the findings from our animal studies and our clinical trials, and will summarise our current understanding of the disruptive effect of GA on the clock and sleep and what might be done about it.

Using the honey bee we have shown that GA (2% isoflurane for six hours) can effectively cause 'jet lag' in bees, shifting the circadian clock to a different time zone by acting on the expression of the core genes (*period* and *cryptochrome*) that drive daily rhythms (1). Furthermore, we have shown that the shifting effects of GA can be ameliorated by the administration of bright light during anaesthesia (2). These findings led us to investigate the same effect in more mainstream model organisms: transgenic circadian clock-reporter flies and mice.

Initial clinical studies on the extent of post-operative circadian and sleep disruption were hindered by the fact that disease itself causes sleep and clock disruption. We have recently completed a trial with a unique patient population –40 donor nephrectomy patients- to examine the "real world" effects of anaesthesia and surgery on the clock and sleep, and whether light administered during surgery might counteract GA induced jet-lag in these patients as it does in insects.

(1) Cheeseman, J.F., Winnebeck, E.C., Millar, C.D., Kirkland, L.S., Sleigh, J., Goodwin, M., Pawley, M.D.M., Bloch G., Lehmann, K., Menzel, R., and Warman, G.R. (2016). *General anaesthesia alters time perception by phase shifting the circadian clock.* Proc Natl Acad Sci U S A. 109 (18): 7061-6.

(2 Ludin, N.M., Cheeseman, J.F., Merry, A.F., Millar C.D., Warman, G.R. (2016). *The effects of the general anaesthetic isoflurane on the honey bee (Apis mellifera) circadian clock* Chronobiology International 33 (1): 128-133

S1B.2: The role of PERIOD1 within the central circadian clock network: when evolution lags behind

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Jetlag or desynchronosis, is a temporary disorder of the circadian clock system, a modern-day malaise that accompanied men's invention of the aircraft, and creation of shift work. Desynchronosis can, however, uncover valuable information on the molecular and physiological assembly of the circadian system.

Considering that the clockwork component *Period1* (*Per1*) is an immediate early target gene of the light input pathway to the suprachiasmatic nucleus (SCN), the master circadian clock, we sought to investigate its role in the adaptability of SCN-dependent rhythms to temporal shifts. Accordingly, we used wild-type and *Per1* knockout (*Per1*$^{-/-}$) mice, and analysed their flexibility to entrain to abrupt shifts of the external light/dark (LD) cycle. Our findings show that *Per1*$^{-/-}$ mice subjected to such a jetlag protocol using both LD phase delays and advances (simulating trans-meridian flights across time zones in both directions - east-bound and west-bound), exhibit accelerated phase alignments of physiological/behavioural rhythms to the shifted LD cycles. We further investigated where along the light input pathway PER1 mediates its influence.

While PER1 is thought to be involved in conveying photic information to the central clock in the SCN, our study suggests a regulatory role, in which PER1 contributes to the gradual adaptation of the clock to shifts in local time, and hence the "lag" in jetlag.

S1B.3: Does it matter at what time of day we eat?

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Time-restricted feeding (TRF), i.e. intermittent energy restriction periods recurring daily is a diet strategy with apparent efficacy in promoting weight loss. It is unknown as to whether the timing of the TRF period is critical for maximising its effects. Since metabolism is closely linked to the circadian clock, we investigated whether leptin sensitivity in the hypothalamus is regulated in a circadian manner and whether the beneficial effects of TRF depend on the timing of the feeding period. We established that within the arcuate nucleus, a key brain region for body weight regulation, leptin-induced pSTAT3 (the main transcription factor of leptin) was controlled by a daily rhythm, however, the profile of the rhythm differed depending on diet composition. Surprisingly, molecular leptin sensitivity in mice fed high fat diet (HFD) for 28 days was undistinguishable from mice on low fat diet during the last half of the light phase and first half of the dark phase suggesting that HFD does not lead to leptin resistance universally. Furthermore, also the anorexigenic effect of leptin was dependent on the timing of application and diet composition. Surprisingly, the daily rhythm of caloric intake between mice on LFD and HFD was identical over the 24 hour rhythm except elevation of caloric intake in HFD mice between ZT 9-3 (3h of light and 3 h of dark period) independent of activity. TRF (a limitation of HFD to a 6 hours continuous period, daily for 3 weeks) regardless of daily timing led to a marked reduction in food intake and body weight but not in locomotor activity or energy expenditure compared with ad libitum fed mice. TRF at ZT 9-3 led to reduced food intake and plasma insulin compared with other time-periods. These data suggest that TRF may be a promising weight loss strategy and depends on circadian timing.

S1B.4: Regulation of the GnRH neuronal network by circadian output neuropeptides

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Fertility is controlled by a neuronal network converging on gonadotropin releasing hormone (GnRH) neurons. In female rodents, the GnRH surge triggering ovulation occurs at the end of the proestrous afternoon. Precise timing of the surge relies on daily signals arising from the central circadian clock in the suprachiasmatic nucleus (SCN) that are relayed to the GnRH neuronal network via projections (i) from SCN vasoactive intestinal peptide (VIP)-producing neurons to GnRH neurons and (ii) from SCN arginine vasopressin (AVP)-producing neurons to preoptic area (POA) kisspeptin neurons, upstream of GnRH neurons.

We combined electrophysiology and calcium imaging in brain slices from mice in which GnRH and POA kisspeptin neurons express the green fluorescent protein or the genetically encoded calcium indicator GCaMP3 to examine the actions of SCN-derived neuropeptides on these neurons. VIP and AVP stimulated the activity of GnRH and POA kisspeptin neurons, respectively. Effects of VIP on GnRH neuron activity and of AVP on POA kisspeptin neurons required activation of VIP receptors and type 1 AVP receptors, respectively. Because the surge is generated only on the proestrous late afternoon, we tested whether the effects of SCN-derived neuropeptides on GnRH and POA kisspeptin neurons varied in a time of day- and an estrous cycle-dependent manner. Surprisingly, effects of VIP on GnRH neurons and AVP on POA kisspeptin neurons were similar in the hours leading up to, or at the expected time of the proestrous surge. Moreover, these responses were not different to those found on diestrus.

These data reveal that SCN neurons may drive GnRH and POA kisspeptin neuron activity through activation of VIP and AVP receptors, respectively. However, our results show that the functioning of SCN neuropeptide receptors on GnRH and POA kisspeptin neurons cannot account for the gating of the GnRH surge to the end of the proestrous afternoon.

S2A.1: microRNA based investigations into the biology of aortic valve stenosis

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Aortic valve stenosis (AS) is a major cause of morbidity and mortality worldwide, with no effective medical therapies. Investigation into the underlying biology of AS in humans is limited by a lack of accepted preclinical models, and difficulties in obtaining healthy human valvular tissue for use as a control group. However, micro-ribonucleic acids (miRNAs) are stable in post-mortem tissue, allowing us to compare valve specimens from patients undergoing aortic valve replacement for AS to non-diseased cadaveric valves.

We found 106 differentially expressed miRNAs ($p < 0.05$, adjusted for multiple comparisons) on microarray analysis, with highly correlated expression among up- and down-regulated miRNAs. Integrated miRNA/gene expression analysis validated the microarray results as a whole, while quantitative polymerase chain reaction confirmed downregulation of miR-122-5p, miR-625-5p, miR-30e-5p and upregulation of miR-21-5p and miR-221-3p. Pathway analysis of the integrated miRNA/mRNA network identified pathways predominantly involved in extracellular matrix function. A number of currently available therapies target products of upregulated genes in the integrated miRNA/mRNA network, with these genes being predominantly more peripheral members of the network.

To investigate the connection between AS disease stage and circulating miRNA profile, we performed microarray-based whole miRNome profiling of 24 participants with aortic stenosis and 27 control participants. After adjustment for age and multiple testing, we identified four miRNAs significantly different between groups. These findings were then examined using quantitative polymerase chain reaction in a larger validation cohort of 101 controls and 94 participants with AS, stratified in a prespecified analysis by presence of co-existing coronary artery disease, with mixed results.

In conclusion, while we found that circulating miRNA profiling is not yet suitable for clinical use in AS, miRNA-based approaches are useful in elucidating the underlying biology of diseases that are otherwise difficult to investigate.

S2A.2: MicroRNAs in cardiac homeostasis and injury

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After it was first discovered that only a very small portion of the human genome codes for genes involved in protein synthesis it soon became apparent that other more complex regulation of protein expression occurs. MicroRNAs are small nucleotide sequences (~22) that can target mRNA to suppress synthesis of a specific protein or initiate mRNA degradation. To date microRNAs are reportedly highly conserved across species, although it is unclear whether each has an identical function in all species and which are actually active at one time due to stoichiometric competitive inhibition. With over 28,000 now annotated in the latest miRBase (release21), many are yet to be validated functionally. However, it is apparent that microRNAs act as exquisite regulators of complex multifactorial tissue-specific processes that underpin homeostasis in a large range of developmental, maintenance and adaptive cellular processes, from stem cell differentiation and proliferation, to cell survival and death signalling processes. Thus it is not surprising that microRNAs are also implicated in a large variety of diseases, including congenital and acquired heart disease. Consequently, changes in microRNA profile expression or their extracellular release have been examined for potential as clinical biomarkers. In addition, select microRNAs have been targeted in therapeutic strategies to drive specific cellular response. Major challenges reside in progressing microRNA methodology, including assay, quantification, *in vivo* validation, bioinformatic analyses of multiple microRNA changes, and implementation of microRNA manipulation and modulation. In this presentation key microRNA expression changes identified in cardiac and vascular responses to cardiac disorders and injury are reviewed from human and experimental animal models. Defining microRNAs and their function provides new mechanistic insights into cellular processes underpinning health and disease.

S2A.3: A novel oligonucleotide-based drug targeting VE-Cadherin as a new therapy for inflammatory disease

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Inflammatory diseases are characterised by dysfunction of blood vessels with one of the hallmarks being vascular leak. Vascular endothelial cell cadherin (VE-cadherin), is a major endothelial cell-specific junctional protein that controls vascular integrity. A decrease in its expression, increased shedding or presence of anti-VE-cadherin antibodies is associated with many inflammatory diseases and of cancer. We have identified the microRNA, miR-27a, a member of the miR-23-27-24 polycistronic complex, as a direct regulator of VE-cadherin. Interestingly, mir-27a is increased in diseases such as solid tumours, diabetic retinopathy and cerebral cavernous malformations (CCM). We have developed an oligonucleotide-based inhibitor (CD5-2) that binds to the 3'UTR of VE-cadherin at the miR-27a binding site and blocks the binding of mR-27a but fails to engage the Argonaute complex. Treatment of endothelial cells with CD5-2 results in increased VE-cadherin expression and inhibition of permeability. CD5-2 has profound effects on diseases where vascular leak is a central feature, namely solid tumour growth, diabetic retinopathy and CCM. Our work establishes a role for VE-cadherin as a novel target and offers a preclinical proof of concept for the first-in-class drug, CD5-2, as a therapeutic modifier of blood vessels.

S2A.4: Using microRNAs to predict heart disease events in the general population

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MicroRNAs (miRNAs) are small regulatory RNAs that are stable in plasma and have potential clinical utility as diagnostic and prognostic biomarkers in heart disease. We have previously shown that miRNAs are prognostic in coronary heart disease patients, and now we have investigated whether circulating miRNAs may also predict risk of near future cardiovascular events, in asymptomatic individuals from the general population.

We performed an initial pilot study comparing plasma levels of 10 miRNAs in individuals who subsequently experienced a cardiovascular event < 12 months, with those who remained event-free > 5 years (n=71). We then tested selected miRNAs (miR-127-3p, miR-146a-5p and miR-223-3p) in a separate group of individuals (n=95), to confirm their association with incident cardiovascular events and assess whether they provide new prognostic information independent of current cardiovascular risk scores and established cardiac markers. Lastly, we tested the specificity of these miRNAs for atherosclerotic disease in patients with a spectrum of cardiovascular and non-cardiovascular disease.

In our pilot study, higher levels of miR-223, miR-127 and miR-146a were associated with increased risk of cardiovascular events ($p<0.01$). In our validation study, we confirmed the association for mir-223, but not miR-127 or miR-146a (event rate for miR-223-3p tertiles: 37%, 67%, 68%, $p=0.021$). This association was independent of the Framingham risk score ($p=0.014$) and high sensitivity troponin I ($p=0.014$), but not the cardiac biomarker, NT-proBNP ($p=0.203$). However, miR-223-3p was not specifically associated with atherosclerotic heart disease, as levels were correlated with C-reactive protein, a general marker of inflammation ($p\leq0.002$), and also elevated in chronic obstructive pulmonary disease patients ($p=0.009$).

Our data suggest that miR-223 is associated with inflammation and increased cardiovascular risk in asymptomatic individuals, but is not specific for atherosclerotic heart disease and is unlikely to improve risk prediction beyond established cardiac markers.

S2B.1: Quantifying ventilation and perfusion in the human lung with contrast-free proton MRI

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The use of proton magnetic resonance imaging (MRI) in the lung is hampered by the low signal levels present in the lung due to its expanded structure, and by the rapid signal decay caused by magnetic susceptibility effects resulting from the numerous air-tissue interfaces inherent in the structure. However, the absence of any form of ionizing radiation or other form of dose-limitation proffers the advantage of repeated imaging. Arterial Spin Labeling is used extensively in studies of cerebral blood flow and has been successfully adapted for use in the lung. By including well-characterized phantoms in the images, quantitative images of blood flow can be obtained. Ventilation can be imaged indirectly by exploiting the T1 effect of oxygen in lung tissue. By tracking the rate of equilibration of a T1-weighted image following a switch of inspired gas from air to 100% oxygen, the regional specific ventilation can be measured. Combining this with the quantitative lung density determined from dual echo time gradient echo technique provides actual ventilation. The combination of these imaging techniques allows the regional determination of ventilation-perfusion ratio, the determinant of the efficiency of pulmonary gas exchange. In addition the repeatability of the techniques provides the ability to repeatedly study subjects, allowing physiological or treatment interventions, and permitting the examination of spatial-temporal dynamics in blood flow and ventilation.

S2B.2: Casein Kinase 1δ/ε inhibitors: a new class of anti-asthma agents?

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Asthma affects more than 10% of the Australian and New Zealand population. Severe steroid-dependent asthma occurs in ~5% of patients but is estimated to account for 50% of the cost. The only mechanistically new therapies for asthma in the past 30 years, target so-called T2 asthma, which is driven by T helper 2 – type cytokines, with antibodies targetting interleukin-5 or its receptor. These biological therapies do not meet the needs of the majority of the severe steroid dependent asthma subgroup. Our efforts have been directed to identifying pathways which underlie the remodelling response, which is prominent in severe asthma. This focus on structural cell types within the airway wall has identified that the mechanical microenvironment impacts on the pharmacology of the airway, a phenomenon more generally referred to as mechanopharmacology (Krishnan et al, 2016). Recent work has identified casein kinase 1δ/ε (CK1δ/ε) as a downstream effector of the remodelling and steroid resistance inducing effects of transforming growth factor β (Xia et al 2017). This CK1δ/ε pathway is also implicated in regulating the circadian clock "signalling circuit" via phosphorylation of CLOCK repressors, period and cryptochrome. The connection of CK1δ/ε to glucocorticoid activity holds the potential to explain the well-known connection between circadian rhythm and severity of allergy and asthma symptoms. Which are more pronounced in the early morning. We are now examining the clinical development potential of the CK1δ/ε inhibitor, PF670462 in treatment of severe asthma.

Krishnan, R., Park, J-A., Seow, C.Y., Lee, P.V.S., Stewart, A.G. (2016). Cellular biomechanics in drug screening and evaluation: Mechanopharmacology. *Trends in Pharmacological Sciences* 37:87-100.

Xia, Y.C., Radwan, A., Keenan, C.R., Langenbach, S.Y. Li, M Radojicic, D., Londrigan, S.L, Gualano, R.C., Stewart, A.G. (2017). Glucocorticoid insensitivity in virally infected airway epithelial cells is dependent on transforming growth factor-β activity. *PLOS Pathogens* http://dx.doi.org/10.1371/journal.ppat.1006138

S2B.3: Clinical usability assessment – The long haul

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Abstract: Fisher & Paykel Healthcare has recently completed a large scale product development project updating one of the company's flagship medical devices. The focus was to optimise device usability. The product development process incorporated design to reduce user error and user frustration, and testing to challenge this, culminated in a multi-centre clinical usability assessment. During the clinical assessment use and device performance trends were monitored providing a feedback look for iteration to the final design. An overview of the usability assessment process, the feedback we received from nurses and doctors, and some of the design iterations that were implemented as a result, will be presented.

S2B.4: Sustainable life cycle respiratory product development

Geoff Bold[1]

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Abstract: Fisher & Paykel Healthcare is a leading designer and manufacturer of products used for respiratory care applications. We sell our products in more than 120 countries worldwide and employ over 3,500 people around the world including more than 500 staff dedicated to research and development. With such a global reach the environmental impact of our products is being considered more and more. How can we innovate to design products and their life cycles to address sometimes apparently conflicting requirements of being clean and safe for patients, and yet environmentally sustainable.

S3A.1: Oxygen consumption for work and other processes in right ventricular hypertrophied papillary muscle

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Mechanical efficiency (work for pumping/oxygen consumption) of the right ventricular (RV) myocardium of pulmonary hypertension patients decreases with disease severity[1]. A similar decrease was found in right ventricular papillary muscles of rats in which pulmonary hypertension was induced by monocrotaline (60 mg/kg), and work during sinusoidal length changes (5 s^{-1}) and oxygen consumption were measured in an oxygen chamber at 37°C[2]. The cross-bridge inhibitor blebbistatin (10 µM) was used to determine whether oxygen use for cross-bridges or other processes was increased. Quantitative enzyme histochemistry was used to investigate mitochondrial function in the experimental muscle (volume typically 0.5 mm^3) and RV wall. The hypothesis was tested that reduced efficiency is due to release of cytochrome c. Monocrotaline-induced PH increased myocyte cross-sectional area by 65%. Blebbistatin reduced power output completely. Oxygen uptake for cross-bridges was proportional to power output ($r = 0.87$, $P<0.001$, $n =14$). Mechanical efficiency in hypertrophied papillary muscles (range 22 to -2%) correlated with oxygen uptake after blebbistatin ($r = -0.61$, $P = 0.02$, $n=14$). Oxygen uptake after blebbistatin was reduced by 50 µM ruthenium red by only 17 (SE7)%. Oxygen uptake after blebbistatin correlated with the activity of complex I ($r = -0.84$, $P = 0.005$, $n=9$), but not with the cytosolic cytochrome c concentration. Cytosolic cytochrome c correlated with the proton permeability of the mitochondrial innermembrane ($r= -0.88$, $P<0.001$, $n=13$). Mitochondrial complex II, IV and V activities were similar to control. In the RV myocardium cardiolipin metabolism changed, and glucose-6-phosphate dehydrogenase and MAO-A activities increased. It is concluded that mechanical efficiency in experimental PH is predominantly reduced due to low ATP/O_2 by mitochondrial changes, oxygen use by MAO-A and NADPH oxidation.

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2. Wong YY, Handoko ML, Mouchaers KTB, de Man FS, Vonk Noordegraaf A, van der Laarse WJ (2010). *Reduced mechanical efficiency of rat papillary muscle related to degree of hypertrophy of cardionyocytes.* Am J Physiol Circ Physiol 298: H1190-H1197

S3A.2: The role of the myofilament in diabetic left ventricular dysfunction

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The cardiac response to exercise/stress is attenuated by diabetes. Autonomic dysregulation affects both resting and peak heart rate, limiting heart rate reserve. Stroke volume is also reduced in people with diabetes; caused by poor ventricular filling and possibly reduced contractility during exercise. This presentation will describe the cardiovascular exercise responses of people with diabetes and examine the potential role of autonomic dysregulation, impaired diastolic filling and contractile responses to exercise intolerance in diabetes. The talk will finish with our ongoing studies which aim to determine whether altered contractile properties of myofilaments from diabetic human cardiomyocytes contribute to reduced cardiac reserve in diabetes.

S3A.3: Cardiac myometry in health and disease

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The mechanical and energetic performance of cardiac muscle is widely studied *in vitro* using ventricular trabeculae – small linearly-arranged collections of myocytes. Our Cardiac Myometer is a unique instrument that allows us to measure, simultaneously, the intracellular calcium release, force-production, sarcomere length, shape-change, and heat-output of cardiac trabeculae as they undergo contraction and shortening.

Recently, we have developed and implemented two new experimental techniques that allow us to track the internal shortening patterns of a trabecula as it does work against a parameterised mechanical load, mimicking the impedance of the vasculature. Using newly-developed image processing techniques, we can now track the strain and motion of internal structures to nanometre resolution, along the entire length of the specimen and throughout the entire time course of a twitch. Using a real-time hardware implementation of a Windkessel-style model of the vasculature, we can reproduce the time-varying and disease specific mechanical loads experienced by the trabecula *in vivo*, in health and disease.

These tools now allow us to mimic a wide variety of healthy and diseased loading conditions, while studying the macroscopic and microscopic stress and strain performance of the trabecula. Our recent results have revealed a significant degree of non-uniformity in strain and stress during “isometric” contraction. We have further discovered that our novel loading scheme allows trabeculae to develop higher work output and efficiency than has been possible using previous experimental work-loop protocols.

S3A.4: Left-ventricular energetics in diabetes and in hypertension

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Diabetes and systemic hypertension are both leading risk factors for heart failure. These diseases place abnormally high workloads on the left ventricle. In response, the left ventricle enlarges, initially undergoing compensated hypertrophy but ultimately suffering end-stage decompensated failure. In contrast, in pulmonary hypertension, the left ventricle experiences a reduced workload and hence undergoes atrophy. We assessed the energetics of the left ventricle of rats induced with either one of these three diseases in order to partition the effect of hypertension from the effect of hypertrophy. Experiments were performed at the working-heart level and at the trabecula level. Using our unique suite of recently-constructed instruments, we varied the metabolic demands of the preparations by challenging them to perform pressure-volume or force-length work against an extensive range of afterloads. Oxygen consumption or heat production was simultaneously measured, which allowed us to quantify the energy efficiency. The results show distinct differences in energetic performance between the heart and its trabeculae, as well as between diabetes (without hypertension) and hypertension (systemic or pulmonary, where the left ventricle suffers hypertrophy or atrophy, respectively). We propose several underlying mechanisms that not only proffer reconciliation of these differences, but also provide deeper insights into cardiac energetics in diseases.

S3B.1: Early life nutrition and the path to programmed obesity – can GH intervention make a difference?

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Maternal undernutrition (UN) during pregnancy results in cardi-metabolic dysfunction in offspring. This includes obesity, insulin resistance and low-grade inflammation. Maternal UN is known to cause alterations in the growth hormone-insulin like growth factor (GH-IGF) axis in offspring. It is now clear that intervention strategies during critical periods of developmental plasticity have the ability to reverse some of the adverse consequences associated with early life programming. However, many of these models focus on nutrition and lifestyle strategies. Given the importance of GH to somatic growth and development we utilised a rat model of moderate maternal UN to examine the effects of pre-weaning GH treatment on metabolic outcomes in offspring. Maternal UN resulted in increased adiposity, elevated systolic blood pressure, endothelial dysfunction, impaired insulin sensitivity and a pro-inflammatory phenotype in adult offspring. These effects were reversed in UN offspring treated with GH in the pre-weaning period. Many of these effects were specific to offspring of UN mothers and may therefore offer a targeted intervention strategy to reverse metabolic and cardiovascular dysfunction arising as a consequence of early life maternal undernutrition.

S3B.2: The function of growth hormone beyond growth

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GH is produced after cessation of growth and is the most abundant hormone in the adult pituitary gland. In the adult, it regulates the metabolic process and the integrity of many tissues. Adults who lack GH develop a characteristic clinical picture of metabolic, body composition and functional abnormalities. These patients exhibit insulin resistance, hyperlipidaemia and increased levels of pro-inflammatory cytokines, collectively increasing the risk of cardiovascular mortality. Body fat is increased while muscle and bone mineral are reduced. Cardiac function, muscle strength and physical fitness are impaired. Psychological function and quality of life are adversely affected.

GH replacement reverses metabolic and body composition abnormalities resulting in improved physical and psychological function. GH status should be re-evaluated in the transition age for continued treatment to complete somatic development. It is not yet known whether cardiovascular mortality is returned to normal. Up to twenty years of experience reveals that the benefits of GH replacement are sustained, that GH is safe with no evidence of increased risk of tumour recurrence or of de novo malignancy.

Adults with GH deficiency have impaired health, which improve with GH treatment. Adults with GH deficiency should be replaced with GH.

S3B.3: Physical exercise improves age-associated cognitive deficits in a growth hormone-dependent manner

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Advancing age results in alterations for multiple systems and regions within the brain with many undergoing decline. This includes decreases in neural stem cell number, neurogenesis rate and cognitive function. Aerobic exercise has been shown to be a low cost, non-invasive approach whilst being one of the most potent stimulators of neurogenesis in both young and old animals. It is now accepted that the hippocampus of the adult brain, an area important for learning and memory, contains a population of dormant precursor cells that can be activated to produce more neurons. We have recently shown that an optimal period of physical exercise activates these cells in very aged mice. Interestingly, we also demonstrate that these changes correlate with an increase in circulating growth hormone (GH). Importantly, aged animals that have undergone optimised voluntary physical exercise demonstrate improved cognitive function in the form of spatial memory and learning. Addition of GH to a purified population of neural precursor cells resulted in a significant increase demonstrating a direct effect on the precursor cell population. Finally, exercise-dependent improvements in aged animals can be mimicked via pharmacological induction of GH release. By defining a possible mechanism involved in exercise-mediated improvements it may be possible to develop effective strategies to combat age-associated cognitive deficits.

S3B.4: Contribution of human growth hormone to cancer progression

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