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Conference Report

Abstracts of the 2023 Autumn Meeting of the British Society for Cardiovascular Research †

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† Presented at the 2023 Autumn Meeting of the British Society for Cardiovascular Research, Wadham College, University of Oxford, Oxford, UK, 4–5 September 2023.

Abstract: The Autumn Meeting of the British Society for Cardiovascular Research in 2023 was organized by Carolyn Carr, Lisa Heather and Claudia Montes Aparicio at Wadham College at the University of Oxford and was the 50th Anniversary Meeting of the Society. The theme of the meeting was "The impact of dysregulated metabolism on cardiovascular function" and included an early career symposium on "Life in academia and beyond". The Annual Bernard and Joan Marshall Distinguished Investigator Lecture was given by Professor Doug Lewandowski on "Metabolic flux in the driver's seat during cardiac health and disease". This paper presents the abstracts selected for oral and poster presentation.

Keywords: cardiovascular research; immuno-metabolism; heart failure; metabolic flux

1. Selected Oral Abstracts

1.1. Microtubule-Associated Protein 1S (MAP1S) Regulates Cardiomyocyte Viability through Modulation of Autophagy and Apoptosis

Pia Morales, Yulia Suciati Kohar, Gina Galli, Sukhpal Prehar, Min Zi, Elizabeth Cartwright, Ashraf Kitmitto and Delvac Oceandy
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Introduction. Autophagy and apoptosis are both essential for cardiomyocyte homeostasis and heavily involved in cardiac remodelling following myocardial infarction (MI). Remarkably, it was recently discovered that microtubule-associated protein 1S (MAP1S) interacts with of a key autophagy marker, LC3. Here, we investigate the role of MAP1S in regulating cardiomyocyte autophagy and apoptosis.

Methods and results. Overexpression of MAP1S in neonatal rat cardiomyocytes (NRCM) was achieved using adenoviral vector. To determine autophagy flux, we used GFP-LC3-expressing adenovirus. We observed a significant increase in the number of GFP-LC3 puncta in MAP1S-overexpressing NRCM compared to controls after treatment with rapamycin and chloroquine, suggesting a higher autophagy flux in cells overexpressing MAP1S. On the other hand, overexpression of MAP1S significantly reduced apoptosis and increased survival of NRCM in response to H_2O_2-induced oxidative stress, as confirmed by TUNEL and MTT assays. We next examined whether ablation of MAP1S in mice affects cardiac phenotype following MI. We subjected MAP1S global knockout (KO) mice to LAD ligation and found significantly higher mortality in MAP1S-KO mice (60%) compared to wild-type counterparts (30%) 4-week post-MI. Interestingly, cardiomyocyte apoptosis was significantly more elevated in KO mice than wild-type controls (11.3% vs. 23.8%). To determine whether transient overexpression of MAP1S leads to enhance survival and protection of cardiomyocytes against apoptosis, we synthesized modified mRNA as a mode of gene delivery of MAP1S and injected it into the myocardium after LAD ligation in C57BL/6J mice. These data are currently being analysed and will be presented at the conference.

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Conclusion. Our findings suggest that MAP1S plays a role in cardiomyocyte survival by regulating autophagy and apoptosis. However, more research is needed to reveal the molecular mechanism implicated in this protective effect.

1.2. Progression of Diabetic Cardiomyopathy Exhibits Alterations in Cardiac Function and Structure and Macrophages Composition

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Background: Diabetic cardiomyopathy (DbCM) is defined as a cardiac dysfunction derived from diabetes mellitus (DM) without the presence of coronary artery diseases and hypertension. However, there are no specific treatments for DbCM, and the uniqueness of DbCM remains controversial. Therefore, this study aimed to understand the characteristics of DbCM using longitudinal follow-up echocardiography and single-nuclei RNA sequencing.

Method: DbCM murine model was established using a high-fat diet (HFD) and a single dose of 100 mg/kg streptozotocin (STZ). Echocardiography was used for longitudinal follow-up of cardiac function and structure. Single-nuclei RNA sequencing (snRNA-seq) was performed to decipher the progression of DbCM. Then, pathway analysis was conducted, along with RT-qPCR and Western blotting.

Results: HFD/STZ mice recapitulated the DbCM phenotype, particularly diastolic dysfunction and left ventricular hypertrophy, after 24 weeks of follow-up. Interestingly, the MV E/A ratio significantly decreased in HFD/STZ mice at 8 weeks of study ($p < 0.05$) compared to controls. Left ventricular posterior wall thickness was increased in HFD/STZ mice ($p < 0.0001$). The results of snRNA-seq showed an influx of macrophages into diabetic hearts, and the dysregulated inflammatory pathway was one of the critical drivers in DbCM progression. Moreover, the chronic hyperglycaemic condition resulted in induced interferon regulatory factor 7 (Irf7) gene expression, which is a stress-inducible factor. Gene expression of Irf7 showed significantly higher levels in diabetic heart tissue vs. controls ($p < 0.01$) and high (D-glucose)-glucose vs. osmotic control (L-glucose)-treated pro-repairing macrophages ($p < 0.01$).

Conclusion: Diabetic cardiomyopathy mice reveal multifaceted characteristics, including diastolic dysfunction, cardiac hypertrophy, and immune inflammation in the heart. These suggest therapeutic targets for modulating diabetic cardiomyopathy progression by immunomodulatory therapies.

1.3. Fumarate Reprogrammes Metabolic Preferences and Antioxidant Profile within the Cardiomyocyte

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Fumarate is a Krebs cycle intermediate involved in energy generation in the heart. There is increasing evidence that metabolites can also serve signalling roles within the cell and regulate the response to ischemia. In cancer, fumarate has been shown to act as a signalling molecule, so we questioned whether there was a role for fumarate in regulating cardiomyocyte biology and metabolism.

Perfusing rat hearts exposed with hypoxic buffer caused a 3.5-fold increase in intracellular fumarate. Culturing human iPSC-derived cardiomyocytes (hiPSC-CM) in hypoxia (2% O_2) caused an 54% increase in fumarate release into the media. Culturing hiPSC-CM with the cell-permeable fumarate derivative dimethyl fumarate (DMF) promoted the nuclear translocation of the transcription factor Nrf2 and upregulation of antioxidant genes. In addition, DMF metabolically reprogrammed cardiac substrate utilisation by decreasing genes involved in fatty acid metabolism and upregulating glucose metabolism genes. This

resulted in decreased palmitate oxidation rates and increased glycolytic rates and increased lactate production, measured using radiotracers. Changes in the mitochondrial network morphology were detected using confocal microscopy, but this was not associated with changes in mitochondrial respiration assessed using the Seahorse analyser. Finally, FRET experiments and in silico modelling demonstrated that fumarate and fumarate derivatives can activate Gi-coupled signalling pathways to decrease intracellular cAMP concentrations.

In conclusion, fumarate is an intracellular metabolite that selectively accumulates during hypoxia and can act as a signalling molecule activating transcription factors and modulating metabolism. Fumarate can also be released from the cardiomyocyte to act as a paracrine signal via G-protein coupled receptors. Taken together, fumarate may act to reprogram hypoxic cells and their borderzone neighbouring cells in response to myocardial ischaemia.

1.4. Targeting Mitochondrial Succinate Transport for the Prevention of Cardiac Ischemia/Reperfusion Injury

Hiran A. Prag [1], Jiro Abe [2], Laura Pala [3], Sophia Schanz [1], Stuart T. Caldwell [3], Richard C. Hartley [3], Michael P. Murphy [2] and Thomas Krieg [1]

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Background: The ischemic accumulation of the mitochondrial metabolite succinate and its rapid oxidation by succinate dehydrogenase (SDH) upon reperfusion drives ischemia/reperfusion injury (IRI) in myocardial infarction. Succinate oxidation produces reactive oxygen species (ROS) by reverse electron transport (RET) at mitochondrial complex I, which orchestrates a cascade of events leading to cardiomyocyte death. During ischemia, the mitochondria-produced succinate is transported into the cytosol, enabling the accumulation of a large succinate pool. On reperfusion, the succinate is transported back into the mitochondria and oxidised by SDH; thus, we hypothesised that targeting succinate mitochondrial transport could be a novel therapeutic target to prevent cardiac IRI.

Methods: Isolated heart mitochondria, cultured H9C2, C2C12 myocytes, and primary cardiomyocytes were used to assess the impact of inhibitors on respiration, RET-ROS production, inhibitor uptake, and succinate metabolism. LAD ligation and reperfusion in C57BL/6J mice was used as an in vivo MI model.

Results: Butylmalonate, a mitochondrial dicarboxylate carrier (DIC) inhibitor, blocks succinate-dependent respiration and RET-ROS in mitochondria, but its entry into cells is inefficient and it has little impact in vivo. We developed novel butylmalonate esters to improve butylmalonate delivery to cardiomyocytes in vivo. We found that diacetoxymethyl butylmalonate (DAB) effectively delivered butylmalonate and could prevent succinate accumulation and oxidation in vitro. DAB could also prevent succinate-mediated respiration and RET-ROS at lower concentrations than butylmalonate alone. Finally, DAB reduced acute infarct size in vivo when administered at reperfusion.

Conclusions: Blocking succinate transport back into mitochondria at reperfusion prevents the damage in IRI by preventing the oxidation of succinate by SDH and downstream RET-ROS production and may be a promising novel strategy for preventing cardiac IRI.

2. Selected Poster Abstracts

2.1. Inappropriate Sinus Tachycardia in Post-COVID-19 Syndrome Patients. Does Dysautonomia Play a Role?

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Background: Inappropriate sinus tachycardia (IST) is a prevalent manifestation in patients with post-COVID-19 syndrome (PCS), impeding them from performing their regular activities.

Aims: This retrospective study aims to investigate the prevalence of IST and its accompanying cardiovascular symptoms in PCS patients. Additionally, the present study evaluates cardiac autonomic function through heart rate variability (HRV) analysis to investigate the role of dysautonomia in the development of IST in the context of PCS.

Methods and results: A total of 306 PCS patients (female: 228, male: 78) were selected and divided into two age- and gender-matched groups to undergo a comparative sub-analysis: group 1, PCS patients with IST (n = 68, female: 55, mean age = 42.13 $\pm$ 10.59 years), and group 2, PCS patients without IST (n = 238, female: 173, mean age = 42.05 $\pm$ 9.50 years). Every subject underwent 24 h Holter monitoring prior to the study, and the electrocardiogram (ECG) recordings were analysed for time- and frequency-domain HRV parameters. As a result, 22.22% of the PCS patients presented IST. There were significant HRV alterations in the IST group compared to the IST-absent group. The LF/HF ratio was significantly larger in the IST group (2.80 $\pm$ 1.93) compared to the IST-absent group (2.31 $\pm$ 2.25, p = 0.02). While the sympathetic nervous system (SNS) index was significantly higher in the IST group (1.60 $\pm$ 0.68 vs. 0.01 $\pm$ 0.61, $p < 0.001$), the parasympathetic nervous system (PNS) index was significantly lower in the IST group (-1.84 ± 0.40 vs. -0.61 ± 0.78, $p < 0.001$).

Conclusion: Cardiac dysautonomia in PCS patients with IST shifts the cardiac sympathovagal balance towards increased SNS activity and decreased PNS activity, with a greater influence on the PNS component. This might be key in the pathogenesis of IST in the context of PCS. A thorough understanding of the autonomic modifications in PCS would yield more effective screening and treatment methods to address IST.

2.2. The Impact of Dysregulated Systemic Metabolism on Cardiovascular Function in Experimental Chronic Kidney Disease

M Young [1], F Cullen [1], N Patel [1], A Vujic [2], J Clark [3], L Karatzia [1], MM Yaqoob [4] and D. Aksentijevic [1]

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Chronic kidney disease (CKD) affects 840 million people worldwide and is one of the leading causes of mortality. Nevertheless, for most CKD patients, the risk of developing cardiovascular disease is higher than progression to kidney failure. The aetiology of cardiac complications in CKD is multifactorial and remains to be elucidated. Using two models of CKD, this study aimed to examine whether the systemic metabolic alterations and cardiac remodelling in CKD are causally linked. In this study, 5/6 nephrectomy (9, 12 weeks, n = 7–9) and adenine-diet-feeding (0.75%, n = 10) CKD was induced in Wistar rats, systemic metabolomic profile analysed using 1H NMR spectroscopy (liver, skeletal muscle, kidney) and enzymatic assays (plasma). Cardiometabolic profile was assessed in vivo (echocardiography) and ex vivo (Langendorff perfusion) at baseline and after 20 min ischemia. Both models of CKD were characterised by significant renal dysfunction (creatinine, $p < 0.05$), anaemia (haematocrit, $p < 0.05$) and alterations in exogenous substrate homeostasis (glucose, fatty acids, insulin, creatine kinase). Whilst CKD models at baseline were characterized by HFpEF (CKD 78% EF vs. control 75%, n = 16), the systemic metabolic milieu consisted of severe, distinct metabolic risk clusters in the liver, kidneys, and skeletal muscles. This altered systemic bioenergetic reserve predisposed CKD hearts to worse functional outcomes after 20 min ischemia (CKD 13% vs. control 34% LVDP recovery n = 5–7). Thus, prevention of systemic metabolic derangement may be a new approach to ameliorate remodelling in CKD cardiomyopathy.

2.3. Tracking Glucose Metabolism in Mice after Physiological Exercise Training

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Rationale: Exercise induces beneficial whole-body adaptations, but the significance of metabolic remodelling in exercise-induced physiological changes is unclear [1]. In this study, we investigated glucose metabolism in exercise-trained mice.

Methods: Female C57BL/6J mice underwent 4 weeks of voluntary wheel running (n = 7) or remained sedentary (n = 7). $^{13}C_6$-glucose (1 mg/g body weight) was intraperitoneally injected after the last exercise session, and tissue samples were collected 20 min later. Metabolites were analysed using ultra-high-performance liquid chromatography/mass spectrometry [2] and statistically evaluated with multiple t-tests (5% FDR correction).

Results: In vivo glucose isotope tracing showed no significant changes in ^{13}C incorporation in cardiac glycolytic and TCA cycle intermediates at rest. However, exercise-trained gastrocnemius muscle exhibited increased ^{13}C incorporation in TCA cycle intermediates, particularly cis-aconitate (p = 0.04), with marginal increases in fumarate (p = 0.05) and malate (p = 0.07). Kidney TCA metabolites, including citrate (p = 0.032), malate (p = 0.090), and aspartate (p = 0.060), displayed elevated ^{13}C incorporation after exercise training. Notably, exercise-trained mice showed higher ^{13}C incorporation of the ketone body BHB in the heart (p = 0.048) and kidney (p = 0.090), while liver BHB ^{13}C incorporation remained unchanged.

Conclusion: Resting hearts of exercise-trained mice did not exhibit significant changes in ^{13}C incorporation in glycolytic and TCA metabolites, whereas increased TCA flux was observed in the gastrocnemius muscle and kidney together with enhanced BHB utilization in the heart and kidney. These findings provide insights into the metabolic remodelling across various organs in response to exercise training and offer insights for further molecular regulation studies.

2.4. Endothelial Interferon Signaling May Represent a Key Mediator of Adverse Cardiac Remodelling Associated with Experimental Diabetes

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Objective: Diabetic cardiomyopathy (DCM) is a significant complication of type 2 diabetes, which is characterized by dysfunction of the coronary microvascular endothelium and adverse cardiac remodelling. Although many signalling pathways are linked with DCM, specific mediators remain poorly understood. Therefore, this study aimed to identify key signalling mechanisms underlying endothelial dysfunction in DCM as potential therapeutic targets.

Methods and Results: Ingenuity Pathway Analysis (IPA) was performed on data obtained from both bulk and single-nuclei RNAseq analyses of mouse DCM tissue using R and Partek. Data sets met the criteria of an adjusted p-value of 0.05 and a log2 fold change ranging from −1.5 to 1.5, focusing on differentially expressed genes with endothelial cell enrichment. Interferon signalling and activation of interferon-related factors were identified as the most significantly altered canonical pathways linked with endothelial dysfunction, with expression of several component genes found to be significantly upregulated within the dataset, including interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), IFIT3, and interferon-stimulated gene 15 (ISG15), which were validated by real-time RT-PCR analysis of DCM mouse tissue. Parallel in vitro studies established treatment of human coronary microvascular endothelial cells (HCMECs) with 25 mmol/L D-glucose for 14 days (versus L-glucose osmotic control) as a reliable model of experimental diabetes, characterized by barrier dysfunction (increased FITC-Dextran transfer, reduced expression of e.g., ZO-1, claudin-5, β-catenin) and significantly increased protein expression of IFIT3 and ISG15.

Conclusion: Taken together, these data indicate endothelial interferon signalling as an interesting focus for investigation as a potential target to reduce progression of experimental DCM.

2.5. Reversing Intracellular Sodium Elevation in Heart Failure: A Novel Optogenetic Approach

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A rise in intracellular sodium ([Na]i) concentration is a hallmark of heart failure (HF) which causes contractile dysfunction and arrhythmias, as well as metabolic reprogramming. These pathological effects suggest that [Na]i would be a viable therapeutic target in HF. However, there is currently no pharmacological method to lower [Na]i once elevated. In 2013, the light driven outward Na pump eKR2 was discovered in marine bacteria by Inoue et al. [3], and this pump has since been used to drive Na efflux in a number of optogenetic studies. Therefore, this study aims to use eKR2 as an optogenetic tool to investigate the therapeutic potential of lowering [Na]i in the failing myocardium. [Na]i was measured in isolated neonatal rat cardiomyocytes using a novel application of the ^{23}Na-NMR spectroscopic method as well as SBFI fluorescence methods. Cells were exposed to a range of hypertrophic agonists (phenylephrine (PE), endothelin-1 (ET-1), angiotensin-II (ATII) and aldosterone (Aldo)). Both PE and ET-1 increased cell surface area, ANP and BNP expression, glucose utilisation, and lactate production in cells after 48 h. At baseline, [Na]i measured using ^{23}Na-NMR in cardiomyocytes was 9.9 $\pm$ 1.6 mM, and this increased by approximately 54% after PE treatment. Cells were then transduced with eKR2-YFP using AAV2.9 and [Na]i measured after 10 days in control and eKR2-YFP cardiomyocytes exposed to 525 nm light. Exposure to 525 nm light (3 mW/mm^2 for 30 min) lowered [Na]i in PE-treated eKR2-YFP cells by approximately 44%. We demonstrate for the first time that [Na]i is elevated in cardiomyocytes exposed to a variety of hypertrophic agonists using a combination of a novel ^{23}Na-NMR technique and conventional SBFI fluorescence measurements. We also demonstrate that this [Na]i elevation can be reversed by expression and activation of the light-driven Na pump eKR2. This approach allows us to test the hypothesis that lowering [Na]i is a useful therapeutic approach for the treatment of HF.

2.6. Serum Metabolomics Improve Risk Stratification for Incident Heart Failure

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Rationale: The prediction and early detection of heart failure (HF) is crucial to mitigate its impact on quality of life, survival, and healthcare expenditure.

Approach: We explored the predictive value within UK Biobank of nuclear magnetic resonance serum metabolomics (168 metabolites) for incident HF both individually and in combination with existing risk scores (PCP-HF [4], including medical history, physical measurements, and clinical chemistry). We fitted per-metabolite COX-regression models to assess HF associations in 68,311 individuals. Elastic net models were trained on an 80% training split (n = 54,649) and evaluated using the remaining test partition (20%, n = 13,662).

Results: Several metabolites showed independent associations with incident HF (90/168 adjusting for age and sex, 48/168 adjusting for PCP-HF; $p < 0.01$). Elastic net models effectively retained key features representing highly correlated clusters (PCP-HF + metabolomics: 9/12 PCP-HF and 16/168 metabolite features). Adding metabolomics to PCP-HF improved predictive performance (C: 0.755 vs. 0.768, continuous NRI = 0.287, relative IDI: 17.47). Simplified models including age, sex, and metabolomics

performed almost as well as PCP-HF (C: 0.745 vs. 0.755, continuous NRI: 0.097, relative IDI: 13.445). Risk and survival stratification were improved by integrating metabolomics.

Conclusions: Serum metabolomics improve incident HF risk prediction. Scores obtained from age, sex and metabolomics exhibit similar predictive power as clinical models.

Expected value: Offering serum metabolomics to a middle-aged cohort might significantly improve HF risk stratification, thus facilitating preventive efforts. Via a single blood draw, metabolomics might offer a highly cost- and time-effective, standardizable, and scalable alternative to more complex scores. Our machine learning approach accommodates the socioeconomic necessity to reduce the number of measured features whilst preserving performance.

2.7. The Exercise-Mediated Metabokine Beta-Aminoisobutyric Acid Is an Exercise Mimetic Driving Skeletal Muscle Metabolic and Functional Adaptation

Hélène N Daou [1], Nicole T Watt [1], Amanda DV MacCannell [1], Shaimaa Gad [1], Mattia Scalabrin [2], Simon Futers [1], David J Beech [1], T. Scott Bowen [2], Lee D Roberts [1]

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Skeletal muscle integrates many of the systemic signals which contribute to the adaptive remodelling and beneficial effects of exercise. One mechanism through which muscle mediates the systemic effects of exercise is through muscle-derived hormones known as myokines. We identified the metabolite β-aminoisobutyric acid (BAIBA) as an exercise-mediated small molecule myokine. BAIBA is secreted from muscle in response to increased expression of the transcriptional co-regulator PGC-1α, a master regulator of the muscle adaptive response to exercise. However, how BAIBA functions to regulate the adaptive responses of skeletal muscle to exercise remains poorly understood. We show that BAIBA improves muscle metabolism, exercise efficiency, and performance in mice. Oxygen consumption (VO_2) and energy expenditure are increased in BAIBA-treated mice. Furthermore, BAIBA increases soleus in situ muscle contractile force, fatigue resistance, mitochondrial number, and function. We found that BAIBA drives muscle fibre-type switching to an oxidative phenotype in vivo. BAIBA regulates specific fibre-type gene expression in human myocytes through PPARδ. Our findings demonstrate that BAIBA is a key paracrine myokine which, in part, regulates the effects of exercise to improve muscle function with resultant effects on exercise performance.

2.8. Do Plasma Levels of Interlukin-1β Correlate to Cardiac Dysfunction in Coronary Artery Disease?

Lewis Pearson [1], Alicia Staley [1], Bethan Samphire-Noden [1], Courtney Riley [1], Nidal Bittar [2], Vasanthi Vasudevan [2], Sarah Withers [1] and David Greensmith [1]

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Coronary artery disease (CAD) is a leading cause of mortality worldwide, producing an estimated 17.8 million deaths each year [5]. In CAD, inflammation is associated with elevated levels of cytokines such as interleukin-1β (IL-1β) [6]. However, the levels to which these cytokines are elevated in CAD is not clear, nor is the extent to which they responsible for impaired cardiac function. As such, this preliminary study quantified levels of serum IL-1β, then correlated those levels to indices of cardiac function in a CAD patient cohort. The study was conducted in accordance with local and IRAS ethical approval (IRAS ID: 247341). Serum samples were taken from consenting patients scheduled for revascularisation surgery. Levels of IL-1β were measured using a high-sensitivity ELISA kit (Invitrogen, Massachusetts). Average plasma IL-1β concentrations were 0.94 ± 0.013 pg/mL (n = 68). Patient IL-1β levels correlated significantly with peak E-wave velocity (n = 56, r^2 = 0.09, p = 0.03), and strongly, but insignificantly, with E/A ratio

(n = 55, r^2 = 0.2, p = 0.06). We observed no significant correlations with other indices of systolic and diastolic function, including EF (n = 61, r^2 = 0.005, p = 0.6), LVOT (n = 45, r^2= 0.00008, p = 0.95), TAPSE (n = 42, r^2 = 0.01 p = 0.22), and LVIDD (n = 50, r^2 = 0.016, p= 0.4). These preliminary data suggest that serum IL-1β levels may be useful as a diagnostic marker in CAD. Future work will increase the power of the study and investigate the cellular mechanisms that link IL-1β levels with whole-heart dysfunction.

2.9. Do Plasma Levels of Tumour Necrosis Factor (TNFα) Correlate to Cardiac Dysfunction in Coronary Artery Disease?

Alicia Staley [1], Lewis Pearson [1], Bethan Samphire-Noden [1], Courtney Riley [1], Matthew Jones [1], Nidal Bittar [2], Vasanthi Vasudevan [2], Sarah Withers [1] and David Greensmith [1]

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Coronary artery disease (CAD) is a chronic inflammatory condition and associated with high rates of mortality worldwide [7]. Cytokines such as tumour necrosis factor (TNFα) play a key role in the pathogenesis of CAD [8], though the degree to which TNFα is elevated or alters cardiac function remains unclear. Therefore, this preliminary study quantified serum TNFα levels in a CAD patient cohort, then correlated those levels to measurements of systolic and diastolic cardiac function. The study was conducted in accordance with local and IRAS ethical approval (IRAS ID: 247341). Serum samples were taken from consenting patients scheduled for revascularisation surgery. Levels of TNFα were measured using a high-sensitivity ELISA kit. Measurements of cardiac function were extracted from patient clinical records. Average plasma TNFα levels were 2.27 ± 0.35 pg/mL (n = 66). Preliminary data indicate that TNFα levels may correlate with LVIDD, though this did not reach significance (p = 0.06; r^2 = 0.09; n = 61). We observed no significant correlations with other indices of systolic and diastolic function, including EF (p = 0.6993; r^2 = 0.0031; n = 76), LVOT (p = 0.1213; r^2 = 0.0691; n = 59), TAPSE (p = 0.2251; r^2 = 0.0418; n = 55), and E/A ratio (p = 0.1699; r^2 = 0.0434; n = 68). These preliminary data suggest that serum TNFα levels may be of limited use as a diagnostic marker in CAD. However, we now aim to increase the power of the study and explore other correlates. We will also investigate the cellular mechanisms that link TNFα levels with whole heart dysfunction.

2.10. Defining the Role of CALHM2 in Cardiac Metabolic Switching and Development

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Cardiac development is associated with changes in mitochondrial structure and a switch from a glycolytic phenotype to one driven primarily by oxidative phosphorylation. This metabolic switch is essential for myocyte differentiation and the development of adult cardiac structure and function. Work has identified CALHM2 (Calcium homeostasis modulator protein 2) as a gene associated with congenital heart disease (CHD). We have confirmed that CALHM2 is localised within mitochondria and involved in their structure and homeostasis. In addition to their role as the "Powerhouse of the Cell", mitochondria contribute to calcium homeostasis, intracellular signalling, and cell survival. It is therefore entirely unsurprising that defects in mitochondrial structure and function lead to a variety of diseases, many of which are associated with the mitochondria's capacity to perform oxidative phosphorylation. Given the links between CALHM2 in mitochondria and mitochondrial function in cardiac development, work has focused on characterising CALHM2 in embryonic mouse hearts across development and into early adulthood. Going forward, this work will be complemented by studies using a Calhm2$^{-/-}$ mouse to determine the molecular function of CALHM2 in the developing heart and how loss of function leads to CHD. Work has already identified the mitochondrial trifunctional protein (mTFP), a heterodimeric enzyme consisting of ECHA and ECHB subunits, as a potential interacting partner of CALHM2. This complex localises to the inner mitochondrial membrane, where

it facilitates fatty acid β-oxidation. Removing CALHM2 by siRNA knockdown results in reduced localisation of ECHA to the inner mitochondrial membrane; it accumulated in the area surrounding mitochondria, seemingly due to a defect in import machinery. These data suggest that CALHM2 may be responsible for importing of fatty acid oxidation machinery into the mitochondria.

2.11. β-Aminoisobutyric Acid Augments Mitochondrial Oxidative Metabolism to Protect against Skeletal Muscle Dysfunction in Obesity-Induced Insulin Resistance

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Aims/Objectives: This study aimed to explore whether the exercise-induced myokine, β-aminoisobutyric acid (BAIBA), protects skeletal muscle mitochondria from dysfunction in a murine model of diet-induced obesity. We have previously demonstrated that BAIBA increases hepatic b-oxidation and enhances adipose tissue browning to protect against glucose intolerance and weight gain. It is unknown if BAIBA has a paracrine effect on skeletal muscle metabolism.

Methods: C57/Bl6 mice were placed on a high-fat diet (HFD) at 8 weeks of age. At 16 weeks, insulin and glucose tolerance tests (ITT/GTT, respectively) were conducted and animals were allocated to either continue receiving HFD alone or to also receive 100 mg/kg/day BAIBA in their drinking water. After a further 14 weeks, a second ITT and GTT was conducted, and the oxidative mitochondrial metabolism of the soleus muscle from the mice was analysed using high resolution respirometry.

Results: The mice became insulin-resistant after HFD treatment, whereas BAIBA treatment reduced insulin resistance. There was an increase ($p < 0.05$) in muscle oxidation of fatty acid substrates (palmitoyl L-carnitine) in all the HFD animals. However, there was a significant reduction ($p < 0.05$) in mitochondrial function and energy production in the HFD mice compared with normal chow-fed animals. Concurrent BAIBA treatment protected skeletal muscle mitochondria from obesity-induced dysfunction in HFD mice.

Conclusions: BAIBA treatment protects against diet-induced insulin resistance. Diet-induced insulin resistance causes mitochondrial dysfunction in skeletal muscle, which compromises energy production. Treatment with BAIBA augments mitochondrial energy production, which may help to preserve muscle function and improve exercise performance.

2.12. Investigating the Impact of Adipose Tissue on Internal Mammary Grafts in Coronary Artery Bypass

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Coronary artery bypass grafting (CABG) is considered as the gold standard for the treatment of ischaemic heart disease. The internal mammary artery (IMA) is used for grafting in ~90% of procedures. Previously, we have shown that nitric oxide (NO) is important for the anticontractile effect of the surrounding adipose tissue (PVAT) in mesenteric arteries. More recently, a loss of NO has been identified as an early event in 5–15% of CABG graft occlusions [9], and understanding the fatty acid profile of vessels used in CABG may be important in predicting outcome [10]. This study aims to characterise the role of PVAT in IMA contractility and to understand the role of NO in mediating this. The study was conducted in accordance with local and IRAS ethical approval (IRAS ID: 247341). Wire myography was used to characterise the contractility in response to noradrenaline (NA: 10–5M to 10–9M) of IMA and eNOS$^{-/-}$ and wildtype aortas$^{+/-}$ PVAT. NO production in response to NA stimulation$^{+/-}$ Oleic acid was ascertained using Griess technique on adipose tissue of wildtype mice. Early studies show PVAT has an anticontractile effect on IMA in response to NA ($p < 0.05$, $n = 4$). There is a release of NO from adipose tissue stimulated with 10–6M NA compared to basal levels ($p < 0.05$, $n = 3$). This is inhibited by oleic acid (NA vs. NA + Oleic: $p < 0.05$, $n = 3$). In contrast, a significant procontractile

effect of PVAT on wildtype and eNOS$^{-/-}$ mouse aorta in response to NA was observed (WT + PVAT vs. WT-PVAT: $p = 0.006$, $n = 10$; eNOS$^{-/-}$ + PVAT vs. eNOS$^{-/-}$ − PVAT: $n = 0.03$, $n = 10$). We confirmed that PVAT has an anticontractile effect on IMAs. Data suggest that oleic acid, a fatty acid found in IMA, has the capacity to inhibit NO in adipose stimulated with NA. Whether this is significant in patients will be determined. Second, modelling IMA using mouse aorta is not comparable and should not be taken further in our studies.

2.13. Metabolic Dysfunction of the Type 2 Diabetic Heart Is Reversed by Blunting CD36 Palmitoylation

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Cardiac metabolism is altered in type 2 diabetes (T2D) and is associated with impaired cardiac function. The fatty acid transporter CD36 is the primary regulated step in fat metabolism importing fatty acids into the heart. We questioned whether changes in CD36 palmitoylation drive the metabolic dysfunction in T2D and if this is a potential target for therapy. T2D rat hearts had abnormal substrate metabolism, with increased fatty acid oxidation, increased triacylglycerol storage, and decreased glycolysis, as measured using radioisotopes in the perfused contracting heart. In T2D, CD36 translocation to the sarcolemma was increased and accompanied by increased CD36 palmitoylation, the latter confirmed in human insulin-resistant IPSC-cardiomyocytes. We found no differences in APT1 or DHHC5 expression in T2D rats, but increased DHHC4 expression compared with controls. When control hearts were treated with the APT1 inhibitor ML348, we found an increase in CD36 palmitoylation, fatty acid oxidation, and triacylglycerol storage, recapitulating the cardiac T2D phenotype. In contrast, when diabetic hearts were perfused with the DHHC inhibitor cyano-myracylamide, a significant reduction in CD36 palmitoylation was observed with accompanying decreases in fatty acid oxidation and triacylglycerol storage, mirroring the healthy heart. We conclude that, in T2D, there is an increase in CD36 palmitoylation which is associated with redistribution of CD36 to the sarcolemma and increased cardiac fat metabolism. We suggest that targeting palmitoylation of CD36 may provide a mechanism to correct the metabolic dysfunction in diabetes.

2.14. Diet-Induced Obesity Following Myocardial Infarction Increases Skeletal Muscle Fatty Acid Transporter Expression in C57BL/6 Mice

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Patients with heart failure (HF) commonly experience exercise intolerance due to muscle dysfunction [11], which becomes further exacerbated in the comorbidity diabetic heart failure (DHF) [12]. Our previous research indicates extensive muscular dysfunction in DHF models characterised by muscle fibre atrophy and impaired isotonic contractile dynamics in the glycolytic extensor digitorum longus (EDL) [13], consistent with impaired mitochondrial function and quantity [12]. However, the individual molecular phenotype underlying these changes in the EDL remains unclear, with mitochondrial metabolic fuel selection remaining extensively uncharacterised in DHF. We aimed to determine if DHF drives a novel metabolic switch in the EDL from glycolytic to oxidative metabolism. We performed qRT-PCR on EDL samples from mice fed a 10-week, 60% fat HFD following an

MI (DHF) compared to standard chow-fed following an MI (HF), HFD-fed sham (HFD), or standard chow sham control (C) mice. The expression of fatty acid β-oxidation genes Acadm and Acadl was increased in HFD but was not reflected in DHF, indicative of a blunted response to fatty acyl chain length fuel selection. Moreover, DHF mice displayed an upregulation of the cytoplasmic fatty acid transporter Fabp4 and the mitochondrial fatty acid transporters Cpt1a and Cpt2, potentially alluding to increased mitochondrial entry of long chain fatty acids, which we shall confirm functionally by carnitine palmitoyl transferase assays. Overall, our data suggest that at the gene expression level, DHF is sufficient to cause fatty acid transport gene upregulation and may eventually provide rationale to manipulate long-chain fatty acid β-oxidation in DHF.

2.15. Immunophenotyping Tissue-Resident B Cells in Ischaemic Heart Disease by Full Spectrum Flow Cytometry

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Rationale: The immune response is central in atherosclerosis and myocardial infarction (MI). B cell subsets have critical atheroprotective and pro-atherogenic properties, but these mechanisms in complex tissues are poorly understood. Full-spectrum flow cytometry is an emerging technology which has the potential to offer cost-effective high dimensionality analysis but has not previously been optimised for human tissue.

Methodology: Adipose, lymphoid, and vascular tissues are acquired from patients undergoing coronary artery bypass graft (CABG) surgery for acute MI, symptomatic angina pectoris, or non-CABG cardiac/thoracic surgery. Single-cell suspensions of immune cells, stained overnight with antibody-fluorochrome conjugates, were analysed by full-spectrum flow cytometry the following day.

Results: Two 30-colour B cell- and T cell-focussed spectral panels were designed and optimised to comprehensively phenotype the landscape of tissue-resident and circulating immune cells, with complexity indexes of 13.31 and 12.57, respectively. Two-dimensional gating strategies have been applied, alongside dimensionality reduction algorithms, with observed between-group and between-tissue differences in B cell populations.

Conclusions: Spectral flow cytometry has been optimised to immunophenotype complex human tissues acquired during CABG procedures in ischaemic heart disease (IHD) and MI. High-dimensionality flow data have shown B cell population differences between tissue types and disease states. Understanding B cell mechanisms in IHD and MI will guide future targeted immunomodulatory therapies.

2.16. Induction of Experimental Type 2 Diabetes Mellitus Using Combination of High Fat Diet and Multiple Low Doses of Streptozotocin in C57/BL6J Mice Mimics Human Diabetic Cardiomyopathy

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Type 2 diabetes mellitus (T2DM) is a systemic metabolic disorder characterised by an increased risk of heart failure. Diabetic cardiomyopathy (dbCM) can present initially with normal systolic but impaired diastolic function (heart failure with preserved ejection fraction (HFpEF)) [14]. Whilst a combination of a high-fat diet (HFD) and multiple low doses of freshly injected streptozocin (STZ) has been widely used for T2DM induction [15], emerging data show that a combination of HFD and anomer-equilibrated STZ produces an early-onset and robust T2DM model [16]. The aim of this study was to develop a stable murine T2DM model analogous to human dbCM and examine the evolution of cardiometabolic dysfunction. Our study compared three groups (C57/BL6J mice): 1. HFD + freshly injected STZ T2DM (n = 7), 2. HFD + equilibrated STZ T2DM (n = 8),

3. Controls (standard chow and citrate buffer vehicle, $n = 12$). In vivo function (echocardiography) and intraperitoneal glucose tolerance tests (IPGTT) were carried out at 4 and 8 weeks. No significant differences in weight loss associated with T2DM development or morphological parameters were observed across groups. T2DM groups were characterised by extensive metabolic phenotyping (1HNMR spectroscopy: skeletal muscle, liver, kidney, LC/MS: hearts). Unlike freshly injected STZ T2DM, the anomer-equilibrated STZ T2DM induction model was characterized by the development of HFpEF and diastolic dysfunction (anomer-equilibrated STZ E'/A' 0.69 vs. control 1.03, $p = 0.0030$, anomer-equilibrated STZ E/E' 45.63 vs. control 32.58, $p = 0.0333$, anomer-equilibrated STZ MV deceleration 27.01 vs. control 17.25, $p = 0.0103$, anomer-equilibrated STZ EF 76.35% vs. control EF 74.13%, $n = 19$). This is the first study to show HFpEF in the HFD and anomer-equilibrated STZ T2DM induction pre-clinical model, which could serve as a useful future tool for the study of T2DM.

2.17. Phoenix Dactylifera Extract Improves Metabolic and Cardiac Remodeling in Experimental Diabetic Cardiomyopathy

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Diabetic cardiomyopathy is characterized by structural and functional alterations in the myocardium promoting heart failure. There are no current specific treatments, although in research based-settings, antihyperglycemic plant-based extracts have shown promising results. The current study aimed to explore metabolic and ventricular remodelling potential of Phoenix dactylifera in rats subjected to diabetic cardiomyopathy. For induction, Wistar albino male rats (1–3 weeks; $n = 24$) were fed with a high-fat diet for 2 months, followed by i.p administration of nicotinamide (110 mg/kg/bw) and streptozotocin (35 mg/kg/bw). Diabetic rats were divided into three groups: Positive control (PC), standard control (SC; metformin @200 mg/kg/bw), treatment group (PD: Phoenix dactylifera extract @5 mg/kg/bw; orally once daily), with comparison to negative controls (NC; $n = 8$) fed on a normal diet. Fasting blood glucose (FBG) and ECG were monitored regularly. After decapitation (days 15 and 30), glucose, lipid profile, total oxidant status (TOS), total antioxidant capacity (TAC), electrolytes, and myocardial enzymes (CK-MB, LDH) were assessed in serum. PD diabetic rats showed significant ($p \leq 0.05$) reduction in FBG (122.5 $\pm$ 5.5 mg/dL), serum glucose (82.5 $\pm$ 6.5 mg/dL), TOS (56 $\pm$ 2 mmol H_2O_2/equiv.), CK-MB (1279 $\pm$ 15 IU/L), LDH (1052 $\pm$ 19 IU/L), and electrolytes (Na + 131 $\pm$ 3, K+ 3.98 $\pm$ 3), whilst HDL-c (32 $\pm$ 1.5 g/dL) and TAC (2.68 $\pm$ 0.01 mmol Trolox/equiv.) were increased compared to the PC group (FBG 317.5 $\pm$ 2.5 mg/dL); serum (glucose 199.5 $\pm$ 4.5 mg/dL; LDH 2100 $\pm$ 19; CK-MB 2100 $\pm$ 15) and (TAC 1.04 $\pm$ 0.04). ECG showed significant restoration of heart rate in the PD-treated group versus the PC group, indicating improved cardiac function. These data suggest that Phoenix dactylifera may indirectly modulate cardiac remodelling by regulating metabolic markers.

2.18. Upregulation of Filamin C and Binding Partners in Models of Heart Failure

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Introduction: Z-disc proteins are essential in maintaining the structure of the sarcomere; mutations in Z-disc proteins can lead to cardiomyopathy and heart failure [17].

Preliminary studies have shown that heart failure models can cause an upregulation of Z-disc proteins.

Methods: Western blotting was used to quantify protein expression. Mouse heart failure models used were: MLP knockout (WT control), transaortic constriction (SHAM control), and angiotensin II treatment (saline control). Human samples were acquired from patients with transaortic constriction and preserved ejection fraction (used as control), patients treated with a left ventricular assist device (LVAD), and tissue from explanted hearts following heart transplantation.

Results: Filamin C protein expression in MLP knockout mice was increased 7-fold and myotilin 24-fold compared to WT, a significant upregulation. Filamin C binding partners Hspb1 and Hspb7 and heart failure markers Myh7, Ankrd1, and FHL1 were significantly upregulated. No upregulation of proteins was found in TAC mice compared to SHAM, and only Hspb7 was upregulated in Angiotensin II-treated samples compared to saline controls. Heart failure markers were not significantly upregulated in LVAD-treated and Heart Transplant patients compared to AOS samples, but mean protein expression was increased between 2- and 3-fold. Likewise, mean Filamin C protein expression was increased 8-fold, but did not reach significance.

Discussion: The MLP KO mouse model reveals upregulation of Z-disc proteins in heart failure. Larger sample sizes and a true healthy control may reveal a significant upregulation of Filamin C and binding partners in samples of human heart failure.

2.19. Changes in Myocardial T2 under Different Gas Mixtures as a Potential MRI Biomarker of Heart Disease*

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Introduction: The MRI parameter T2* is emerging as a novel biomarker of cardiomyopathy [18] and could yield important insights into mechanisms of disease and therapy. Here, we quantified changes in T2* under differing inhaled gas mixtures to give a ratiometric measure of myocardial oxygenation. We then tested this approach in an animal model of hypertension to determine whether cardiac T2* mapping was sensitive to pathology.

Methods: We assessed T2* changes in six control mice (9.4T Bruker MRI, multi-gradient-echo, TE = 1.88 ms, 15-echoes). Gas containing 2% isoflurane was switched from room air to carbogen, to 100%O_2 and back to air. In a second study, control (n = 10), hypertensive (5-week angiotensin(II) infusion n = 10) and hypertensive + angiotensin receptor blocker Losartan-treated mice (n = 10), underwent cardiac T2* mapping in air, and 100%O_2. T2* was quantified in the anteroseptal myocardium.

Results: In control mice, myocardial T2* in air was 11.18 $\pm$ 1.98 ms. Two minutes after carbogen inhalation, T2* dropped to 8.81 $\pm$ 2.71 ms, then climbed to 10.93 $\pm$ 2.53 ms in 100%O_2 before being reduced to 10.30 $\pm$ 2.76 ms when switched back to air. Significant differences in ΔT2* were recorded between air and carbogen and carbogen-100%O_2 (p = 0.014) and carbogen-100%O_2 and 100% O_2-air (p = 0.028). In the hypertensive study, T2* in control mice was 8.62 $\pm$ 2.76 ms in air, increasing to 9.46 $\pm$ 2.63 ms in 100%O_2. The response was greater in ang(II) mice (7.38 $\pm$ 1.68 ms in air, 9.16 $\pm$ 3.31 ms in 100%O_2) and the ang(II) + Losartan group (7.53 $\pm$ 2.29 ms in air, 10.18 $\pm$ 3.31 ms in 100%O_2). ΔT2* at 5 weeks significantly increased from the baseline only in the ang(II) infusion + Losartan group (p = 0.1818).

Conclusion: Cardiac T2* mapping performed under different inhaled gas mixtures allows for evaluation of myocardial response to variations in blood oxygenation. The ratiometric approach removed the inherent variability in T2* measurements and could offer a rapid and sensitive method for assessing myocardial dysfunction.

2.20. The Effect of Low-Dose Interleukin-2 on the T Cell Receptor Landscape in Patients with Acute Myocardial Infarction

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Rationale: Atherosclerosis is characterized by chronic inflammation in plaques. Treatments targeting this inflammation are limited, underpinning the need for a thorough understanding of the underlying immune mechanisms. The role of T cells in atherosclerosis has attracted increased attention, with the T cell receptor (TCR) providing an additional method to explore the functions of T lymphocyte biology in atherosclerosis.

Methodology: The Low-Dose Interleukin-2 (IL-2) in Patients With Stable Ischemic Heart Disease and Acute Coronary Syndromes (LILACS) study successfully used low-dose IL-2 (ld-IL-2) to augment anti-inflammatory regulatory T cell (Treg) numbers [19]. However, the effect of ld-IL-2 on the TCR repertoire remained unexplored. Here, we use 5′ scRNA and TCR sequencing to present detailed analysis of the effect of ld-IL-2 on the TCR landscape from the LILACS trial patients.

Results: Ld-IL-2 increases the number of Tregs and the size of Treg clonotypes, while simultaneously decreasing Treg clonotype diversity. Clonally expanded Tregs were activated relative to their non-expanded counterparts, specifically along antigen presentation pathways. We developed a novel bioinformatic technique using the TCR as a barcode to track T cells subsets across timepoints, finding that acute myocardial infarction results in redistribution of Treg clonotypes towards effector phenotypes, with ld-IL-2 reversing this trend. Clonally expanded Tregs show shifted antigen specificity, which can be linked to viral antigens, and demonstrate distinct antigen–receptor interactions with other immune cells, highlighting possible anti-inflammatory mechanisms.

Conclusions: Ld-IL-2 impacts the subset composition of T cells at the clonotype level, with modulation of key immune ligand-receptor interactions and antigen specificity.

2.21. Cardiac Fibroblast Function Is Regulated by Modulation of Tetranectin Expression with Associated Impact on Cardiac Fibrosis In Vivo

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Impairment of function and structural changes that arise in the heart following various insults, including ischaemia, hypertension, and diabetes, can lead to heart failure, which is a major public health concern. HF is characterised by increases in fibrosis, cardiomyocyte hypertrophy, inflammation, and microvascular remodelling in the heart. Tetranectin is found at high levels in normal serum and in some tissues during development, but not usually in healthy adult tissues. It has an important role in extracellular matrix (ECM) remodelling, which is particularly important in heart failure due to its ability to bind ECM components and has been suggested to have a protective function within the muscle, bone, and the circulatory system. The changes in the myocardium leading to heart failure heavily involve ECM remodelling, and by using human cardiac fibroblasts and mice deficient in tetranectin we wanted to investigate this in more detail. A model of angiotensin II mediated cardiac fibrosis was induced in wild-type and tetranectin knockout mice by subcutaneous angiotensin II infusion for 4 weeks. Cardiac function was assessed in animals by echocardiography and heart tissue was collected for analysis of fibrosis and hypertrophy by gene and protein expression and histological analysis to investigate structural changes. The migratory ability of fibroblasts in vitro was dependent on level of tetranectin protein, with gene knockdown causing a reduced ability to migrate. Angiotensin infusion caused increased blood pressure along with evidence of cardiac dysfunction.

Significant hypertrophy and fibrosis developed with a reduction in fibrotic gene expression induction in tetranectin knockout mice. This study demonstrates that cardiac changes associated with heart failure are modified by tetranectin modulation and that knockout of tetranectin can reduce the degree of cardiac dysfunction and fibrosis.

2.22. Assessing Human Epicardial Reactivation Using a 2D Stem-Cell Model of Myocardial Infarction

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Stem-cell-based regenerative therapy is emerging as a treatment for heart failure, but challenges remain. The epicardium has been identified as a potential solution [20], but the characteristics of its activation following injury remain unclear. Here, we aimed to develop a 2D model of myocardial infarction (MI) in human pluripotent stem cell (hPSC) cardiomyocytes (CMs) using available single-cell RNA sequencing from model organisms to determine the transcriptional hallmarks of re-activated epicardium, and then applied our model to quantitate the RNA expression of these markers in hPSC-epicardial cells (EPIs). The transcriptional signature of injury-activated epicardium was determined by performing differential expression analysis (DEA), comparing infarcted heart epicardium to a sham control using mouse single-cell RNA sequencing data [21] in Seurat. CMs were differentiated from hPSCs in 2D culture, subjected to hypoxia, then imaged with light microscopy. Culture medium was retained, and cells were lysed for RT-qPCR of genes associated with infarction. EPIs were differentiated from hPSCs in 2D culture and exposed to media from hPSC-CMs. Our DEA found that genes related to immunity are upregulated in epicardial cells following injury, supporting an emerging role of the immune response in regeneration [22], and suggesting epicardial–immune cell communication in response to MI. Light microscopy following ischaemia showed an arrest in hPSC-CM contractile activity and evidence of necrosis. RT-qPCR showed upregulation of genes associated with MI (NPPB and ANKRD1), without statistical significance. Further optimisation, including triangulation with troponin measurements, is needed to validate this MI model, which could act as an in vitro platform to investigate MI. RT-qPCR of hPSC-EPIs did not show significant expression changes in genes identified by DEA to suggest concordance of hPSC-EPIs with that of mouse EPIs, with replicates showing significant variance.

2.23. Multiparametric Immunometabolic Phenotyping of the Diabetic Heart

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Rationale: The heart's resident macrophages are known to sustain mitochondrial homeostasis [23], but links between cardiac inflammation and energy deficit in diabetes [24] are incompletely understood.

Objective: To define the links between cardiac function, metabolism, and immunophenotype in the dbdb mouse model.

Methods: Cine MRI for cardiac structure/function; hyperpolarised ^{13}C MRS for cardiac metabolism; flow cytometry for cardiac immune cell characterisation.

Results: Compared to control mice (n = 14), db/db mice (n = 16) had reduced LV mass, with increased ejection fraction ($p < 0.05$). db/db mice had 95% lower cardiac pyruvate dehydrogenase flux in vivo, reflecting reduced glucose oxidation ($p < 0.05$). db/db hearts had a 57% reduction in cardiac macrophage number overall ($p = 0.002$). The re-

maining cardiac macrophages were reprogrammed, being less likely to display resident anti-inflammatory markers and more likely to display markers of increased inflammatory monocyte recruitment ($p < 0.05$).

Conclusions: db/db mice had altered cardiac structure, function, and metabolism coupled with loss and reprogramming of the resident macrophage population. Given that resident macrophages are essential for sustaining normal cardiac energetics, these findings may indicate a new pathway contributing to energy deficit in the diabetic heart.

2.24. Investigating the Mechanisms Underlying the Differential Handling of Saturated and Polyunsaturated Fatty Acids in Human Cardiomyocytes

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Although cardiac and hepatic ectopic fat accumulation promotes cardiometabolic disease development, the mechanisms contributing to fat accumulation in these organs remain unclear. In vivo post-prandial human studies showed, at a whole-body level, that polyunsaturated fatty acids (PUFA) are preferentially partitioned into oxidation pathways, whereas saturated fatty acids (SFA) are preferentially partitioned into intracellular storage pathways. However, the mechanisms driving this differential handling of fatty acids (FA) within the cardiomyocyte remain unclear. As such, we differentiated and matured human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) in media containing 400 μM FA with different ratios of SFA and PUFAs for 7 days, followed by characterisation of substrate metabolism, cell stress markers, and transcriptional responses. Over the 7 days of FA exposure, all groups had similar glucose uptake and lactate production and continued to beat with no visible differences between groups. There was no increase across all groups in media lactate dehydrogenase or BNIP3 expression, markers of uncontrolled cell death and mitochondrial stress, respectively, though there was increased expression of the inducible stress-response protein heat shock protein-90 in hiPSC-CMs matured in PUFA-enriched media. Interestingly, while FA uptake was similar in the PUFA and SFA groups during the first 48 h, there was a significant decrease in FA uptake in all groups during the last 48 h, with a greater decrease in the SFA-enriched group. Finally, transcriptomic analysis was performed to investigate key pathways differentially affected between groups, followed by sub-analysis of how the FA-media composition influenced transcriptional responses. Taken together, these findings may help to inform future dietary and therapeutic strategies for cardiometabolic disease.

2.25. Parallel RNA-Sequencing and Proteomics Establishes Expected and Emerging Features of the Human Diabetic Heart in a Human-iPSC Engineered Heart Tissue Model of Type II Diabetes

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Diabetes affects nearly half a billion individuals worldwide and increases the risk of developing cardiovascular disease (CVD). Therefore, biological models representing the human diabetic heart are needed to understand the disease's pathology and develop new treatments [25]. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) can be used to produce engineered heart tissue (EHT) and have facilitated the study of the adult human heart in vitro [26]. We questioned whether culturing human EHT in "diabetic-like media" would recapitulate changes seen in the type 2 diabetic (T2D) heart [25]. Utilizing multi-omics approaches, we investigated known metabolic pathways disrupted in diabetes in our EHT model and identified novel and emerging mechanisms that could be targeted therapeutically. Parallel differential expression (DE), pathway enrichment, PPI network

analysis, and in silico transcription factor (TF) regulatory inference identified known changes in metabolic proteins, genes, or pathways, validating our EHT model as capturing the human in vivo diabetic phenotype. At the mRNA level, the fatty acid metabolism pathway was positively enriched alongside predicted PPAR-alpha TF regulation, and the rate-limiting glycolytic enzyme HK2 was significantly downregulated, jointly reflecting an expected shift in cardiac substrate utilization towards fats. Oxidative phosphorylation showed uniform positive enrichment in both DE and PPI analysis, but coincided with vastly disrupted mitochondrial protein abundance. This mitochondrial disruption aligns with an identified increase in NRF2 mRNA and the reactive oxygen species pathway. We also identified a reduced abundance of the V type proton ATPase subunit with increased CD36 expression in the diabetic EHT, an emerging mechanism of lipid-induced cardiomyopathy. EHT cultured in diabetic media recapitulates many key pathway changes present in the T2D heart, but also identifies novel pathways not previously associated with the disease. This validates the use of EHTs as a model for studying the T2D heart and for future drug development.

2.26. Investigating a Novel Genetic Cause of Cardiomyopathy

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Cardiomyopathy is a unified term for progressive diseases of the myocardium that result in enlarged, thickened, or stiffened pathophysiology, which, ultimately, disrupts the normal function of the heart. A homozygous pathogenic variant, p.Arg253Trp SLC5A6, was identified in a family whereby two siblings presented with dilated cardiomyopathy. SLC5A6 encodes the Sodium-dependent Multivitamin Transporter, a transmembrane protein that is crucial for facilitating the active transport of three vitamins: biotin, pantothenic acid, and lipoic acid, all of which are critical organic enzyme cofactors required for energy metabolism in the mitochondria. Energy metabolism is a fundamental process in cardiac maintenance, and, as the most metabolically demanding organ in the body, the heart requires a high ATP turnover to achieve efficient contractile function of the myocardium. There is little known about the function of SLC5A6 in the heart; therefore, transgenic mouse models have been generated and various immunohistochemical and protein techniques have been performed to investigate its role. A conditional deletion of Slc5a6 in cardiomyocytes results in the development of cardiomyopathy in mice and abnormal ECG parameters were detected from 6 weeks, followed by sudden death at 20 weeks. Electron microscopy of Slc5a6 cardiac-specific knockout (cKO) mice hearts showed abnormal mitochondria morphology and degradation; thus, it is predicted that mutations in SLC5A6 prevent the transport of mitochondrial enzyme cofactors and underpin the progression to cardiomyopathy. We have shown that vitamin supplementation of Slc5a6 cKO knockout mice prolonged the life of mutant mice, which showed normal ECG traces and a reduced mitochondrial complexity index at 20 weeks. Therefore, this project aims not only to establish the role of SLC5A6 in the heart, but also to investigate whether vitamin supplementation to Slc5a6 cKO mice will prevent mitochondrial defects and delay progression to cardiomyopathy.

2.27. Altered Regulation of Calcium Handling Proteins in an In Vivo Rat Model of Angiotensin II-Induced Hypertension

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Hypertension, a major comorbidity in patients with heart failure with preserved ejection fraction, is associated with cardiac remodelling, dysfunction, and altered calcium homeostasis. However, existing models of study and evidence on the mechanisms driving this form of cardiomyopathy remain contradictory. This study aimed to establish a

relevant hypertensive model and to assess alterations in the expression and activation of calcium handling proteins. Mini pumps containing either saline as a vehicle control or Angiotensin II (Ang II) were surgically implanted into adult male Sprague–Dawley rats for 4 weeks. Body weight and tail-cuff-derived blood pressure measurements were recorded pre-surgery and 1 week and 4 weeks post-surgery. Following treatment, cardiac function was assessed, body and organ measurements were obtained, and cardiac tissue was processed for biochemical analysis. Rats infused with Ang II exhibited an increased heart weight to body weight ratio, evidencing cardiac hypertrophy (control: 0.00342, n = 4; Ang II: 0.00407, n = 6). Blood pressure was significantly enhanced even at 1 week post-treatment (control: 119 mmHg, n = 4; Ang II: 178 mmHg, n = 6). Furthermore, echocardiographic analysis revealed a mild increase in left ventricular contractility (control: 50.57%, n = 8; Ang II: 55.98%, n = 12). Initial biochemical analysis revealed no change in Phospholamban phosphorylation (control: 1.00, n = 8; Ang II: 1.13, n = 12). Also, data revealed an increase in the phosphorylation of the Ryanodine receptor via the calcium-calmodulin-dependent protein kinase II (CaMKII) site (control: 1.00, n = 6; Ang II: 2.49, n = 8), coupled with a trend towards increased CaMKII oxidation (control: 1.00, n = 7; Ang II: 2.33, n = 11; p = 0.0552). These findings evidence that Ang II induced both cardiac hypertrophy and hypertension in vivo. Additionally, ongoing biochemical evaluation suggests a role of CaMKII in mediating these modifications.

2.28. Growth Differentiation Factor-15 Antagonism Leads to Histological Changes in the Myocardium of Irradiated PPP1R15A$^{-/-}$ Mice

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Background: We previously reported a model of severe heart failure that consistently results in cachexia in irradiated mice lacking the endoplasmic reticulum (ER) stress-induced factor PPP1R15A. This was not seen in identically treated wild-type littermates. Heart tissue shows activation of ER stress and the integrated stress response, extensive fibrosis, and pro-inflammatory cytokine expression. Cardiomyocytes in irradiated PPP1R15A$^{-/-}$ mice express high levels of Growth Differentiation Factor-15 (GDF15), an important stress response cytokine and emerging biomarker of cardiovascular disease in humans [27]. Treatment of these mice with a monoclonal antibody against mouse GDF15 prevented cachexia and sustained cardiac function.

Objective: To examine how blocking GDF15 rescues heart function in irradiated PPP1R15A$^{-/-}$ mice.

Methods: Haematoxylin and Eosin and Sirius Red staining were used to assess inflammation and fibrosis and analysed using QuPath-0.2.3. GDF15 and ER stress-induced factor CHOP mRNA expression in heart tissue was detected using Single Molecule In Situ Hybridisation (SM-ISH) by RNAscope and analysed using HALO Image Analysis Software.

Results: In vivo blockade of GDF15 action in PPP1R15A$^{-/-}$ mice was associated with a significant reduction in levels of myocardial fibrosis. In contrast, no significant differences were found in the levels of myocardial inflammatory cell infiltration and GDF15 or CHOP expression in irradiated PPP1R15A$^{-/-}$ mice treated with anti-GDF15 or IgG control. Inflammatory cell infiltration, fibrosis, and CHOP expression were negatively correlated with heart function (LVFS%) in anti-GDF15 treated mice. Inflammatory cell infiltration was also negatively correlated with body weight in these mice.

Conclusion: Blockade of GDF15 is associated with histological changes in the myocardium and could constitute a novel therapeutic option to limit cardiac cachexia and improve clinical outcomes in patients with severe systolic heart failure.

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References

1. Moreira, J.B.N.; Wohlwend, N.; Wisløff, U. Exercise and cardiac health: Physiological and molecular insights. *Nat. Metab.* **2020**, *2*, 829–839. [CrossRef] [PubMed]
2. Wilkinson, D.J.; Brook, M.S.; Smith, K.; Atherton, P.J. Stable isotope tracers and exercise physiology: Past, present and future. *J. Physiol.* **2017**, *595*, 2873–2882. [CrossRef] [PubMed]
3. Inoue, K.; Ono, H.; Abe-Yoshizumi, R.; Yoshizawa, S.; Ito, H.; Kogure, K.; Kandori, H. A light-driven sodium ion pump in marine bacteria. *Nat. Commun.* **2013**, *4*, 1678. [CrossRef]
4. Khan, S.S.; Ning, H.; Shah, S.J.; Yancy, C.W.; Carnethon, M.; Berry, J.D.; Mentz, R.J.; O'Brien, E.; Correa, A.; Suthahar, N.; et al. 10-Year Risk Equations for Incident Heart Failure in the General Population. *J. Am. Coll. Cardiol.* **2019**, *73*, 2388–2397. [CrossRef]
5. Brown, J.C.; Gerhardt, T.E.; Kwon, E. *Risk Factors for Coronary Artery Disease*; StatPearls Publishing: Petersburg, FL, USA, 2023.
6. Mai, W.; Liao, Y. Targeting IL-1β in the Treatment of Atherosclerosis. *Front. Immunol.* **2020**, *11*, 589654. [CrossRef]
7. Sayols-Baixeras, S.; Lluís-Ganella, C.; Lucas, G.; Elosua, R. Pathogenesis of coronary artery disease: Focus on genetic risk factors and identification of genetic variants. *Appl. Clin. Genet.* **2014**, *7*, 15–32.
8. Amin, M.N.; Siddiqui, S.A.; Ibrahim, M.; Hakim, M.L.; Ahammed, M.S.; Kabir, A.; Sultana, F. Inflammatory cytokines in the pathogenesis of cardiovascular disease and cancer. *SAGE Open Med.* **2020**, *8*. [CrossRef] [PubMed]
9. Gaudino, M.; Antoniades, C.; Benedetto, U.; Deb, S.; Di Franco, A.; Di Giammarco, G.; Fremes, S.; Glineur, D.; Grau, J.; He, G.-W.; et al. Mechanisms, consequences, and prevention of coronary graft failure. *Circulation.* **2017**, *136*, 1749–1764. [CrossRef]
10. Bandara, E.M.S.; Edirisinghe, D.I.U.; de S Wanniarachchi, D.D.C.; Peiris, H.; Perera, P.P.R.; Jayakrishan, A.G.; Waikar, H.D.; Sharma, S.K.; Abeysuriya, V.; Chandrasena, L.G. A comparative study on fatty acid profile in selected vessels of coronary artery bypass graft (CABG). *PLoS ONE* **2022**, *17*, e0260780. [CrossRef]
11. Bekfani, T.; Bekhite Elsaied, M.; Derlien, S.; Nisser, J.; Westermann, M.; Nietzsche, S.; Hamadanchi, A.; Fröb, E.; Westphal, J.; Haase, D.; et al. Skeletal Muscle Function, Structure, and Metabolism in Patients With Heart Failure With Reduced Ejection Fraction and Heart Failure With Preserved Ejection Fraction. *Circ. Heart Fail.* **2020**, *13*, e007198. [CrossRef]
12. Garnham, J.O.; Roberts, L.D.; Espino-Gonzalez, E.; Whitehead, A.; Swoboda, P.P.; Koshy, A.; Gierula, J.; Paton, M.F.; Cubbon, R.M.; Kearney, M.T.; et al. Chronic heart failure with diabetes mellitus is characterized by a severe skeletal muscle pathology. *J. Cachexia Sarcopenia Muscle* **2020**, *11*, 394–404. [CrossRef] [PubMed]
13. Espino-Gonzalez, E.; Tickle, P.G.; Benson, A.P.; Kissane, R.W.P.; Askew, G.N.; Egginton, E.; Bowen, T.S. Abnormal skeletal muscle blood flow, contractile mechanics and fibre morphology in a rat model of obese-HFpEF. *J. Physiol.* **2021**, *599*, 981–1001. [CrossRef] [PubMed]
14. Dillmann, W.H. Diabetic cardiomyopathy. *Circ. Res.* **2019**, *124*, 1160–1162. [CrossRef] [PubMed]
15. Nath, S.; Ghosh, S.K.; Choudhury, Y. A murine model of type 2 diabetes mellitus, developed using a combination of high fat diet and multiple low doses of streptozotocin treatment, mimics the metabolic characteristics of type 2 diabetes mellitus in humans. *J. Pharmacol. Toxicol. Methods* **2017**, *84*, 20–30. [CrossRef]
16. Kamli-Salino, S.E.J.; Brown, P.A.J.; Haschler, T.N.; Liang, L.; Feliers, D.; Wilson, H.M.; Delibegovic, M. Induction of experimental diabetes and diabetic nephropathy using anomer-equilibrated streptozotocin in male C57BL/6J MICE. *Biochem. Biophys. Res. Communications.* **2023**, *650*, 109–116. [CrossRef]
17. Wadmore, K.; Azad, A.J.; Gehmlich, K. The Role of Z-disc Proteins in Myopathy and Cardiomyopathy. *Int. J. Mol. Sci.* **2021**, *22*, 3058. [CrossRef]
18. Raman, B.; Tunnicliffe, E.M.; Chan, K.; Ariga, R.; Hundertmark, M.; Ohuma, E.O.; Sivalokanathan, S.; Tan, Y.J.G.; Mahmod, M.; Hess, A.T.; et al. Association Between Sarcomeric Variants in Hypertrophic Cardiomyopathy and Myocardial Oxygenation: Insights From a Novel Oxygen-Sensitive Cardiovascular Magnetic Resonance Approach. *Circulation* **2021**, *144*, 20. [CrossRef]
19. Zhao, T.X.; Sriranjan, R.S.; Tuong, Z.K.; Lu, Y.; Sage, A.P.; Nus, M.; Hubsch, A.; Kaloyirou, F.; Vamvaka, E.; Helmy, J.; et al. Regulatory T-Cell Response to Low-Dose Interleukin-2 in Ischemic Heart Disease. *NEJM Evid.* **2022**, *1*, 1. [CrossRef]
20. Bargehr, J.; Ong, L.P.; Colzani, M.; Davaapil, H.; Hofsteen, P.; Bhandari, S.; Gambardella, L.; Le Novère, N.; Iyer, D.; Sampaziotis, F.; et al. Epicardial cells derived from human embryonic stem cells augment cardiomyocyte-driven heart regeneration. *Nat. Biotechnol.* **2019**, *37*, 895–906. [CrossRef]
21. Wang, Z.; Cui, M.; Shah, A.M.; Tan, W.; Liu, N.; Bassel-Duby, R.; Olson, E.N. Cell-Type-Specific Gene Regulatory Networks Underlying Mu-rine Neonatal Heart Regeneration at Single-Cell Resolution. *Cell Rep.* **2020**, *33*, 108472. [CrossRef]

22. Ruparelia, N.; Godec, J.; Lee, L.; Chai, J.T.; Dall'Armellina, E.; McAndrew, D.; Digby, J.E.; Forfar, J.C.; Prendergast, B.D.; Kharbanda, R.K.; et al. Acute myocardial infarction activates distinct inflammation and proliferation pathways in circulating monocytes, prior to recruitment, and identi-fied through conserved transcriptional responses in mice and humans. *Eur. Heart J.* **2015**, *36*, 1923–1934. [CrossRef] [PubMed]
23. Nicolás-Ávila, J.A.; Lechuga-Vieco, A.V.; Esteban-Martínez, L.; Sánchez-Díaz, M.; Díaz-García, E.; Santiago, D.J.; Rubio-Ponce, A.; Li, J.L.; Balachander, A.; Quintana, J.A.; et al. A Network of Macrophages Supports Mitochondrial Ho-meostasis in the Heart. *Cell* **2020**, *183*, 94–109. [CrossRef]
24. Rider, O.J.; Apps, A.; Miller, J.J.J.J.; Lau, J.Y.C.; Lewis, A.J.M.; Peterzan, M.A.; Dodd, M.S.; Lau, A.Z.; Trumper, C.; Gallagher, F.A.; et al. Noninvasive In Vivo Assessment of Cardiac Metabolism in the Healthy and Diabetic Human Heart Using Hyperpolarized 13C MRI. *Circ. Res.* **2020**, *126*, 725–736. [CrossRef] [PubMed]
25. Kerr, M.; Dodd, M.S.; Heather, L.C. The 'Goldilocks zone' of fatty acid metabolism; to ensure that the relationship with cardiac function is just right. *Clin. Sci.* **2017**, *131*, 2079–2094. [CrossRef]
26. Bruyneel, A.A.N.; Carr, C. 3D functional scaffolds for cardiovascular tissue engineering. In *Functional 3D Tissue Engineering Scaffolds Materials, Technologies and Applications*; Woodhead Publishing: Cambridge, UK, 2018; pp. 305–343.
27. Wollert, K.C.; Kempf, T.; Wallentin, L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. *Clin. Chem.* **2017**, *63*, 140–151. [CrossRef] [PubMed]