Experiments on animals and animal tissues

It is a requirement of the Society that all vertebrates (and Octopus vulgaris) used in experiments are humanely treated and, where relevant, humanely killed.

To this end authors must tick the appropriate box to confirm that:

For work conducted in the UK, all procedures accorded with current UK legislation.

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Authors must tick the appropriate box to confirm that:

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Guidelines on the Submission and Presentation of Abstracts

Please note, to constitute an acceptable abstract, the Society requires the following ethical criteria to be met. To be acceptable for publication, experiments on living vertebrates and Octopus vulgaris must conform with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission.

Abstracts of Communications or Demonstrations must state the type of animal used (common name or genus, including man. Where applicable, abstracts must specify the anaesthetics used, and their doses and route of administration, for all experimental procedures (including preparative surgery, e.g. ovariectomy, decerebration, etc.).

For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details). For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant. In experiments where genes are expressed in Xenopus oocytes, full details of the oocyte collection are not necessary. All procedures on human subjects or human tissue must accord with the ethical requirements of the Society regarding relevant authorisation, as indicated in Step 2 of submission; authors must tick the appropriate box to indicate compliance.

PL03

Establishing Equity in Medicine Admissions - Widening Participation in Physiology Prize Lecture

Simon Cork[1]

[1]Anglia Ruskin University, UK

Across the UK, access to medical school remains challenging for those from non-traditional backgrounds. Applicants from private schools have 1.5x the odds of receiving an offer compared to state school applicants, and applicants from the highest socioeconomic backgrounds make up 75% of entrants. Establishing equity in admissions to medical school serves not only to promote social mobility, but is implicated in improved patient care.

In this Widening Participation in Physiology Prize Lecture, I will explore the barriers to equitable medical school admissions, discuss the educational and societal benefits of recruiting students from diverse backgrounds, and reflect on the impact of the widening participation initiatives we have developed to improve access to medicine.

SA01

Belfast's Professors of Physiology: A Historical Overview

Keith Thornbury[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Ireland

The Chair in Physiology in Belfast was inexorably linked to the training of medical students. The first professor was James Drummond who was appointed to a joint chair in Anatomy and Physiology (and later also Botany!) in 1819. Medical teaching in Belfast predated foundation of a formal medical school, so Drummond's appointment was in a school, Belfast Academical Institution, which still operates today. Drummond then played a key role in founding the Medical School in 1835 and continued until retirement in 1849, when the school was integrated into the newly opened Queen's College Belfast. Hugh Carlile was appointed Professor of Anatomy and Physiology in the new school and continued until 1860, when Peter Redfern took over. Like his predecessors, Redfern was more of an anatomist than physiologist. He produced most of his research output on cartilage in his previous post in Aberdeen. Apparently, when he came to Belfast his teaching duties, for which he was widely revered, took over. Throughout the middle of the 19th century, Physiology gradually began to acquire its own identity, so, with the foundation of the Physiological Society in 1976, it was natural to split Redfern's post when he retired in 1893. Hence, William Henry Thompson became the first Dunville Professor of Physiology, which takes its name from an endowment from the wealthy Dunville family of distillers in Belfast. Thompson was a man of great talent and wide research interests which included studying the effects of 'Peptone' when injected into the circulation, lesions of the temporal cortex, the effect atropine and morphine on urine output and the metabolism of arginine. In 1902, Thompson moved on to Trinity College Dublin and was replaced by Thomas Milroy. Milroy was a quiet man, but a productive researcher, again in diverse areas. He and his brother John, who became Professor of Biochemistry, are commemorated by the Milroy Medal, awarded annually to the top Queen's medical student in Physiology and Biochemistry. Henry Barcroft became the next Dunville Professor in 1935 and with him began a golden age for Physiology in Queen's. He studied human blood flow by venous occlusion plethysmography and this continued with David Greenfield, the next Dunville Professor, when Barcroft left for Thomas's Hospital Medical School in 1948. Under Greenfield, Physiology in Queen's reached its zenith with many of his mentees going on to high profile posts across the world. Examples include 'Darty' Glover (Dean of Medicine in Sydney), Bob Whelan (Vice-Chancellor of the University of Western Australia and, later, of the University of Liverpool) John Shepherd (chairman of the Board of Development at the Mayo Clinic) and Ian Roddie, who became the next Dunville Professor in 1964. Greenfield himself went on to found the medical school in the University of Nottingham. Under Ian Roddie's stewardship, a field of research in lymphatic physiology developed in Queen's, with two of his co-workers becoming, in turn, the most recent Dunville Professors, namely Noel McHale and Graham McGeown, both of whom also served their time on the Committee of the Physiological Society.

SA02

'Exercise Physiology Research at Ulster University: Highlights and Future Directions

Conor McClean[1]

[1]Ulster University, United Kingdom

Exercise is a well-known stressor that disrupts the body's normal physiological state, with the physiology of exercise further concerned with how the body functions and responds to the dynamic challenge of skeletal muscle contractions. Ulster University's Sport and Exercise Sciences Research Institute (SESRI) was established in 2002, and since then, Exercise Physiology research has become a cornerstone of the Institute's research outputs. SESRI's work, to date, has demonstrated how during exercise, cells, tissues and organ systems are affected, initiating diverse physiological responses such as altered gene expression, enhanced vascular function, changes to free radical metabolism, and DNA damage that can all be applied in different contexts. Thus, exercise is a remarkable lens through which normal bodily function can be explored. This presentation will summarise SESRI's research demonstrating the molecular, cellular and systemic responses to exercise (both acutely and following training) that underpin adaptations, which can be harnessed to improve training responsiveness, performance and/or health parameters. Specifically, the presentation will focus on research that has sought to understand how exercise of different types, intensities, and durations can elicit different physiological responses, and the mechanisms through which these effects manifest through oxidative stress and inflammatory related pathways. We have also been concerned how supplements such as a broad range of antioxidants have the potential to modify responses to exercise stimuli, and how the physiological compensations to exercise may be affected by certain environmental factors such as hypoxia. Collectively, our data have been used in basic, applied, translational and clinical contexts such as for the prevention and treatment of chronic diseases. This talk will highlight some of our emerging and future research directions including how circadian principles may be applied to exercise prescription for both health and performance outcomes.

SA03

Teaching Physiology in the Global Village.

Etain Tansey[1]

[1]Queen's University Belfast, United Kingdom

Historically, physiology teaching was content-heavy, dominated by didactic lectures and rote learning. I will discuss how much modern physiology education has departed from this practice.

It is a truth universally acknowledged, that physiology is the cornerstone of medicine, but a notoriously difficult subject to learn. Therefore, a physiology student must be in want of an exceptional teacher. In the past, knowledge was transmitted passively from expert to learner (the so-called "Sage on the Stage"; King, 1993). There was limited consideration of how students learn. A modern, student-centred approach facilitates active, inquiry-based learning (the "Guide on the Side"; King, 1993), including case-based learning, which is integrated into the medical curriculum at Queen's University Belfast (QUB). Research shows that active learning improves conceptual understanding and enhances student engagement (Freeman et al., 2014). Alongside, formative feedback and authentic assessments that emphasise reasoning, integration, and application of knowledge rather than memorisation, train students in key transferable skills required for future employment as part of the global workforce.

The modern physiology educator is increasingly a scholarly teacher. QUB researchers are using evidence to guide practice while preparing learners to apply physiological understanding responsibly across diverse health, environmental, and sociocultural contexts. For example, McGahon et al., (2026) integrated the UN Sustainable Development Goals (SDGs) into physiology education, highlighting how a knowledge of physiology is relevant to a student body who care deeply about the world we live in and its people. This group's work contextualises physiology learning to the societal and global issues of today, including global warming, health inequalities, and aging populations. Roe et al., (2024) describes an innovative arts-meets-science approach to teach hypofertility and behavioural skills (for example, respect, empathy, compassion, and interpersonal skills) to medical students, using final year Drama students as simulated patients. The research found that realistic simulations of doctor-patient interactions felt authentic and emphasised the importance of physiology to patient care, while also embedding "human factors" skills that enhance the overall educational experience.

Digital technologies, the use of which rapidly increased during and after the COVID-19 pandemic, have further reshaped physiology education into a "global village". Collaboration between institutions is easier, and shared curricula and co-teaching can be supported across borders and time zones. Virtual physiology laboratories and simulations have become more widespread, particularly in regions where laboratory infrastructure, funding and/or time is limited. More recently, learning analytics and artificial intelligence offer opportunities for a personalised learning experience no matter where you learn. The increased volume and velocity of information now enables rapid, worldwide interaction which the education community is harnessing. However, this hyper-connection comes not without its challenges. Our unpublished research suggests that educators struggle with the "always on" nature of work post-COVID-19 and the blurring of professional boundaries.

In summary, the physiology educators of 2026 must prepare students for a rapidly changing world where critical thinking and problem-solving are essential to the application of physiology to current and future global challenges.

SA05

Gut peptides in the therapy of obesity and diabetes

Peter Flatt[1]

[1]School of Biomedical Sciences, Ulster University, United Kingdom

Recent years have witnessed an explosion in new therapies for type 2 diabetes. For a long time, the key tools for management of the disease were diet/exercise, metformin, sulphonylureas and thiazolidenediones. The preference for orally active small molecules continued with introduction of inhibitors of enzymes DPPIV and SGLT2. However, a new era of peptide therapeutics was heralded by the discovery of the stable GLP-1 mimetic, exendin-4 in the saliva of Gila monster lizard plus its exploitation for diabetes as twice daily injectable. Further structural refinements of human GLP-1 to convey enzyme resistance by N-terminal modification and extended half-life by acylation seeded new generations of GLP-1 mimetics with greater effectiveness that extended to substantial weight loss. Concurrent realisation by diabetologists that Roux-en Y gastric bypass could induce diabetes remission by orchestrating substantial changes in circulating gut hormones spawned enthusiasm for combination therapy or development of unimolecular multi-agonists harnessing the diverse actions of GLP-1, GIP and glucagon. This lecture will chart this journey from perspective of innovative research conducted at Ulster over the past 25 years. It will assess where we are now in terms of our own research and what the future holds for new generations of peptides therapies for obesity-diabetes.

SA06

Physiology approaches in Cancer Research: Unlocking New Targets to Improve Therapy

Karen McCloskey[1]

[1]Queen's University Belfast, United Kingdom

At Queen's University Belfast, physiology education and research has built a legacy of discovery science with translation in health and disease. Application of physiology research approaches to cancer research has identified novel targets with the potential to enhance therapy and improve outcomes. This presentation will highlight two of our research programmes: 'radiotherapy-induced bladder dysfunction' and 'ion channels and cancer'.

Radiotherapy aims to maximise tumour control and minimise adverse effects on neighbouring normal tissue. Despite innovations that more precisely deliver radiation to tumours, many patients experience side-effects from normal tissue toxicity. In pelvic cancers, radiotherapy can evoke acute radiation cystitis and bladder dysfunction which often resolves over time. Late effects are less common, occurring months to years post-radiotherapy, but cause significant and irreversible bladder dysfunction. Investigation of bladder physiology after irradiation is uncovering the underlying pathophysiological mechanisms. CT image-guided radiation of mice bladders recapitulated the clinical context where around 50% of mice exhibited altered voiding patterns with frequent, small volume voids. Interestingly, contractility of bladder tissue strips was changed after irradiation, whether or not voiding patterns were changed. Neurogenic-contractions were smaller in the acute phase, 2 weeks post-radiation and while they increased over subsequent months, did not fully resolve. Impaired contractility was not explained by aberrant voltage-gated Ca^{2+}-activity as depolarization-mediated contractions were unaffected by irradiation. Muscarinic-contractions were also unaffected; therefore, pre-synaptic mechanisms may be radiation-sensitive and further work is needed to better understand this (1).

Ion channels are linked with many cancer hallmarks, with altered mRNA and protein expression of diverse ion channels across many cancers vs. normal tissue commonly reported (2). Interestingly, further changes are detected following cancer treatment. Specialist physiology techniques are necessary to reveal whether these changes translate to aberrant ion channel signalling which contributes to cancer growth, treatment resistance and metastasis. Such work can indicate whether ion channel drugs, used in the clinical management of other conditions, could have therapeutic benefit for cancer.

We, and others, have shown that upregulation of the L-type Ca^{2+}-channel, CaV1.3, encoded by *CACNA1D*, is common in prostate cancer, tracking with Gleason grade and metastasis status. Hormone therapy conditions, via androgen deprivation or androgen receptor inhibition, further upregulates *CACNA1D* expression, sustains CaV1.3 overexpression and may enhance localisation at the plasma membrane. Of note, CaV1.3 ion channel activity could only be detected during hormone therapy conditions with patch-clamp electrophysiology or Ca^{2+}-imaging (3). This suggests that CaV1.3 activity, emerging during hormone therapy, when proliferative capacity is reduced may facilitate adaptation and

survival of treatment-resistant cells that eventually drive recurrence. It remains to be seen whether repurposing calcium channel blockers could be of therapeutic benefit during hormone therapy.

The presentation will share how using physiology lenses in cancer research can foster collaborative, interdisciplinary working that may ultimately improve patient outcomes and quality of life.

SA07

Forty years in Physiology goes by in a flash!

Mark Hollywood[1], Gerard Sergeant[1], Keith Thornbury[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Ireland

Nearly four decades have passed since I first walked through the doors of Queen's University Belfast in September 1986 to study Physiology as an undergraduate. Since then I've been fortunate to build a career alongside outstanding colleagues, united by a shared curiosity about how smooth muscle generates and shapes electrical signals to drive physiological function.

A central theme of the research in our Smooth Muscle Research Centre has been the characterization of ion channels and their role in regulating the electrical and mechanical activity of lymphatic[1-4], lower urinary tract[5-15] and airway smooth muscles[16-19]. Earlier in my career, this led to a significant technical and scientific challenge: the study of lymphatic smooth muscle. At the time, few scientists had successfully recorded electrical activity from freshly isolated lymphatic smooth muscle cells. Nevertheless, we established reliable cell isolation procedures and managed to utilise patch clamping to characterise the major ionic currents in lymphatic smooth muscle[1-4] and lay the foundations for future work on how electrical activity governed lymphatic contractility.

Around the same time, we demonstrated for the first time that interstitial cells of Cajal (ICC), well established as pacemaker cells in the gastrointestinal tract, were also present in non-gastrointestinal smooth muscles[6-10]. This finding expanded the conceptual framework of how smooth muscle contractile activity was generated and coordinated. Subsequent work, supported by NIH funding, explored the electrophysiological properties of these cells and their modulation by neurotransmitters, helping to establish ICC and related cell types as key intermediaries in smooth muscle signalling across multiple organ systems.

We were also fortunate to be actively engaged in translational collaborations with industry. In the early 2000s, in partnership with Andor Technology, we were glad to help in the development of electron multiplying CCD (EMCCD) cameras for biological imaging[20-22]. Originally designed for astronomy, these sensors transformed the ability to detect low Ca^{2+} signals in single cells and tissues, overcoming limitations of earlier imaging systems. Since their commercial introduction in 2003, EMCCD technology quickly became a cornerstone of live-cell imaging, illustrating the impact of cross-disciplinary innovation.

More recently our work has focused on the development of novel ion channel modulators and how they interact with their targets at the molecular level. Through the synthesis and characterization of a novel family of GoSlo-SR compounds we have generated potent ion channel modulators[23-25], determined their molecular mechanism of action[26] and patented the most promising structures[27].

Complementing this in 2020, we identified the LINGO family of proteins as previously unrecognized regulatory subunits of BK channels[28-31]. Given the upregulation of LINGO proteins in Parkinson's disease[28], we have proposed a mechanistic link between LINGO-associated BK channel dysfunction and the emergence of Parkinsonian tremor, opening new avenues for therapeutic intervention. In this invited talk, I'll focus on some of our more recent research in which we've examined the molecular mechanisms underlying BK channel modulation by these novel regulatory LINGO subunits.

SA08

The impact of misalignment with the external light-cycle on diabetic retinopathy

Eleni Beli [1], Hanagh R Winter[1], Varun Pathak[1], Jasenka Guduric-Fuchs[1]

[1]Queens University Belfast, United Kingdom

Circadian disruption can be experienced when one lives in a misalignment of their internal clocks with the environmental light cycles. In this study, we investigated whether a form of circadian disruption experienced by extreme chronotypes impacts the progression of diabetic retinopathy.

Ins2 Akita, hyperglycemic, male mice and healthy controls, at two months of age, were housed for 4 months in a forced desynchrony conditions within the limits of entrainment with light cycles (T22.5 and T27 cycles) that resemble circadian disruption of late and early extreme chronotypes correspondingly. Eye disease endpoints were assessed with in vivo retinal imaging (fundus imaging, OCT), ex-vivo immunohistological approaches for acellular capillaries and vascular morphology, and retina tissue was used for mRNA sequencing. Two Way ANOVA (diabetes, chronotype) was used to identify statistically significance ($p<0.05$)

Retinal thickness was significantly reduced in diabetes by 7% compared to controls and was further reduced by another 7% in both forced desynchrony conditions. The number of acellular capillaries in diabetes was increased by 48% in the diabetic mice undergoing forced desynchrony with effects on the intermediate and deeper vascular layers, where significantly reduced vessel area, vessel length and increased E-lacunarity. The mRNA sequence of the retina confirmed that forced misalignment in diabetes impacted the retina vasculature more than in control. Genes related to one carbon metabolism were among the top genes mostly affected by forced misalignment in diabetes, while a more inflammatory pathway activation was also observed. We further validated a particular target with immunofluorescence, its regulation by the clock and its effect on ednothelial cell physiology in vitro.

Overall, a misaligned light schedule to the internal clock that simulates the social jet lag experienced by extreme chronotypes leads to an acceleration of the retinal structure and microvascular dysfunction observed in diabetes. The effects are manifested due to circadian clock disruption and particularly affect the stress responses to the misalinged light. Circadian disruption could therefore be a modifiable risk factor for prevention of diabetic retinopathy.

SA09

The Hidden Order Controlling Endothelial Function

Matthew Lee[1], Ruaridh Clark [1], Charlotte Buckley[1], Xun Zhang[1], Calum Wilson[1], John McCarron[1]

[1]University of Strathclyde, United Kingdom

The endothelium forms a vast, distributed interface between blood and tissue that continuously integrates mechanical and chemical signals to regulate virtually every cardiovascular function. Endothelial function has traditionally been viewed as arising from a largely uniform population of cells that respond in parallel to external stimuli. Vascular function, it is proposed, reflects the averaged output of equivalent cellular units, with each endothelial cell behaving as a scaled-down representation of the average response of the population. However, this view struggles to explain how coherent vascular responses emerge reliably across space despite pronounced cellular heterogeneity, multiple different simultaneous functional outputs and complex signalling environments. We combine high-resolution calcium imaging with network-based analyses to reveal that endothelial behaviour is governed not by individual cells, but by the structure of their interactions. We show that endothelial signalling forms a highly-organised network characterised by small-world and scale-free properties. Within this architecture, distinct subpopulations of cells play specialised roles. Some act as connectors, linking different groups of cells and integrating signals across the network. Others act as hubs, exerting strong influence within highly coordinated regions. These features enable rapid, robust, and spatially-coordinated signalling to occur. The features also introduce non-linear signalling dynamics, where the relationship between input and output is not proportional but depends on the state of the network. Local changes in activity can be amplified or suppressed through interactions in highly-connected regions. Similar inputs are unaltered in less connected or fragmented areas. As a result, identical stimuli can produce markedly different outcomes depending on how signals are distributed and integrated across the network. This non-linearity gives rise to emergent signalling, where system-level responses arise from the collective interactions of cells rather than from the behaviour of any individual component. Signals are not simply transmitted, but are reshaped, amplified, or dampened by the network through which they propagate. As a result, endothelial responses reflect the organisation of connectivity rather than the properties of individual cells alone. Function, therefore, emerges dynamically from patterns of interaction, enabling coordinated, adaptive and independent multifunctional responses across space. These findings reveal that endothelial behaviour is governed by distributed control arising from local interactions that collectively generate system-level function. Furthermore, they shift the focus from single-cell mechanisms to network architecture as the key determinant of function, stability, and vulnerability in endothelial control. In this framework, there is no central controller; instead, vascular responses arise from a form of collective, network-based intelligence. We propose that the hidden order controlling endothelial function lies in its network architecture, so redefine control as an emergent property of connectivity.

C01

Busting the Myth of 70Kg Man Through Participation in an Anthropomorphic Data Practical

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Historically the discipline of Physiology has had 70Kg "Physiologic" man as a foundational reference model representing an assumed average individual from which "normal" values could be derived (Phillips, 2023). While this may prove useful, the limitations of the concept of "average" man have become more apparent recently, especially in the context of medical education and research with a diverse and global cohort of students, subjects and patients. Students who do not see themselves in the curriculum do not learn as effectively, (Ahmet, 2010; Koens et al., 2005) and the reliance on the male standard promotes a gendered stereotype of physiologic knowledge (Cheng & Yang, 2015).

To challenge the "Physiologic Man" model, we have designed a "Busting the Myth of 70Kg Man" practical to illustrate the wide variability in physiologic data across populations and challenge the myth that a "typical" human exists. Students measure height, weight, body fat percentage, stretch, jump and endurance performance in a practical class. As well as gaining experience in measuring human variables, participants get the opportunity to interpret data and draw inferences from raw numbers. That way, the skills of interpolation are inculcated as well as physiologic principles. The remit of the practical also includes issues around professionalism, informed (and immediately withdrawable) consent, anonymising data, bodily autonomy of volunteers, and awareness that the measurement of some variables (weight and body fat percentage) can be triggering.

After gaining ethics committee approval, a mixed methods approach was used combining both electronic survey data and focus group discussions from 2nd Year Medicine (Exercise and Applied Physiology Student Selected Component (n=14) and Science (Human Biology Degree Programme (n=4) students. The survey assessed students' knowledge of and opinions on the importance of inclusion in the physiology curriculum and their lived experience of completing the practical (both as investigator and subject). Changes in attitudes on undertaking the practical were evaluated with pre- and post- practical Likert questionnaires that calculated responses to statements about the classes from 1 (Strongly disagree) to 5 (Strongly agree). Differences between groups were tested for significance using a Wilcoxon signed rank test. In addition, open questions supplemented the Likert semi-quantitative data, allowing students to express opinions and relate experiences. Focus groups were also conducted to give further opportunity to students to relay their experiences. Thematic analysis of the focus group transcripts and answers to open questions allowed us to develop themes using the framework of Braun and Clarke (2006).

Students disagreed with the contention that 70Kg man was an appropriate reference point for teaching physiologic norms (Mean (±SEM) Likert scores: Female=1.7±0.83, Male=2.2±0.4, Non Binary (NB) =1±0), with slightly more men than women thinking the model was appropriate. Similarly, males felt themselves

better represented in the physiology curriculum ((Mean (±SEM) Likert scores: Male=3.8±1.2, Female=2.1±0.4, NB=2±0). Students found the experience of the practical profoundly affecting, gaining a regard for the importance of communication, emotional safety and the primacy of consent when gathering these data. Students especially stressed the importance of the instructor's language and the tone of respect thus set.

C02

Investigating the Mechanisms Underlying Cardiomyopathy in Diabetes Mellitus Using iPS-Derived Cardiomyocytes

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Introduction

Type II diabetes mellitus is a rapidly growing worldwide health challenge. Diabetic cardiomyopathy is a heart muscle disease characterised by progressive contractile dysfunction independent of coronary artery disease or hypertension. It is driven by metabolic dysfunction, oxidative stress, calcium mishandling and mitochondrial impairment. Classical disease models are limited in their ability to replicate human-specific disease mechanisms. Therefore, we used induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) to provide a strong patient-specific model for investigating cellular and molecular changes in diabetic cardiomyopathy, as they retain donor-specific metabolic and epigenetic characteristics.

Methods

iPSCs generated from three diabetic and three non-diabetic donors were differentiated into cardiomyocytes through temporal modulation of Wnt/β-catenin signalling. The iPSC-derived cardiac cultures contained a heterogeneous population of cardiomyocytes (CMs) and cardiac fibroblasts (CFs), better recapitulating the native myocardial microenvironment. Molecular and functional comparisons were performed using immunofluorescence, RT-qPCR, Western blotting, RNA sequencing, and live-cell fluorescence assays. Functional phenotyping included measurements of mitochondrial reactive oxygen species (ROS), apoptosis, and Ca^{2+} dynamics.

Results

Diabetic iPSC-CMs exhibited increased oxidative stress, elevated apoptosis, and a marked increase in hypertrophic markers associated with cardiac stress, including ANP, BNP, GATA4, and ANKRD1, as well as fibrotic markers including TGFB1, CTGF, FN1, POSTN, COL1A1, and COL3A1. Flow cytometry revealed increased forward scatter (FSC) in diabetic TNNT2+, confirming a hypertrophic phenotype. Furthermore, higher FSC-A of vimentin+ and PDGFRα+ populations, along with a higher proportion of α-SMA-positive cells, indicated increased cardiac fibroblast proliferation and myofibroblast activation. Calcium imaging revealed profound defects during excitation–contraction coupling, characterised by elevated baseline cytosolic Ca^{2+}, reduced sarcoplasmic reticulum Ca^{2+} stores, and delayed Ca^{2+} reuptake, consistent with impaired diastolic relaxation.

RNA sequencing of iPSC-CMs from three donors per group identified 1,412 differentially expressed genes. Pathway enrichment and gene ontology analyses revealed significant upregulation of gene networks related to cardiac hypertrophy, fibrosis, ECM remodelling, and ion channel regulation in diabetic iPS-CMs. The WNT/β-catenin and TGF-β signalling pathways were prominently enriched, both of

which serve as key drivers of maladaptive hypertrophic and fibrotic remodelling in the diabetic cardiac phenotype.

Pharmacological inhibition of Wnt signalling partially rescued the diabetic phenotype, reducing oxidative stress and improving calcium handling in contractile cardiomyocytes. attenuating TGFβ signalling, as demonstrated by reduced SMAD2/3 activation and decreased secretion of TGFβ1 and PAI-1, in addition to hypertrophic markers such as secreted ANP.

Conclusion

Collectively, this study shows that donor-specific iPSC-CMs can recapitulate key features of diabetic cardiomyopathy and provides mechanistic insights, linking aberrant signalling pathways to functional impairment. This work highlights the potential of testing donor-specific therapeutic targets and establishes a clinically relevant platform for treating diabetes-associated heart disease.

C03

Gene therapy-mediated modulation of angiopoietin signalling rescues blood flow changes in the diabetic mouse retina

Peter Barabas[1], Thomas Friedel[1], Evan Troendle[1], Roslyn Cassidy[1], Keith Rochfort[2], Robert Wallace[2], Philip Cummins[2], Lara Carroll[3], Balamurali Ambati[4], Reinhold Medina[1], Alan Stitt[1], Timothy Curtis[1]

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Introduction. Diabetic retinopathy is a progressive blinding complication of diabetes mellitus where a decrease in the angiopoietin-1 to angiopoietin-2 ratio, as well as abnormal blood flow is well documented in patients.

Aims. To establish the efficacy of a gene therapeutic approach to improve retinal angiopoietin-1 signalling in streptozotocin-diabetic mice and study the effect on capillary blood flow speed.

Methods. Using a novel, fluorescent microbead-based imaging method, we mapped capillary blood flow speed in the retina of ketamine/xylazine-anaesthetized non-diabetic and diabetic mice, injected intravitreally with vehicle control (PBS), control vector (AAV.CMV-GFP) or a vector carrying a gene for an engineered, stabilized version of angiopoietin-1 (AAV.CMV-COMPAng1). Speed measurements were accompanied by fundus imaging, fluorescence angiography and ex vivo immunohistochemistry to assess retinal status and spatial gene expression profiles.

Results. Mean capillary flow speed was 1.41±0.09 mm/s (N=6). Flow maps showed changes in capillary blood flow decreasing with retinal eccentricity (1.2±0.2 s-1). While the effect of eccentricity (1.1±0.2 s-1) or mean capillary speed (1.24±0.12 mm/s, N=5) did not change significantly, we noted local decreases (0.93±0.09 mm/s, N=5, $P<0.05$) in capillary blood flow speed on the nasal side of the retina at 10 weeks after diabetes induction. Intravitreal injection of the control vector caused an exacerbation of the effects of diabetes, while AAV.CMV-COMPAng1 returned blood flow speed to control levels.

Conclusions. Our findings suggest that focal functional changes of the capillary bed occur in the streptozotocin mouse model and that the Ang1/Ang2-signaling pathway may be a viable alternative or adjunctive therapeutic target to support normal capillary blood flow in the diabetic retina.

C04

Modulation of contractions and cytosolic Ca2+ by cAMP and its downstream effector proteins, EPAC and PKA

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Penile erection is mediated by relaxation of corpus cavernosum smooth muscle (CCSM), primarily driven by cyclic nucleotide signalling. Current therapies for erectile dysfunction (ED) target the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) axis, with phosphodiesterase-5 inhibitors (PDE5Is) enhancing cGMP signalling to promote relaxation. However, 30-40% of patients, particularly those with diabetes or vascular disease, respond poorly due to impaired NO bioavailability and reduced cGMP formation, limiting efficacy [1].

The parallel cyclic adenosine monophosphate (cAMP) pathway represents an alternative target, traditionally attributed to protein kinase A (PKA)-mediated reductions in intracellular Ca^{2+}. Clinically, this is exploited via intracavernosal prostaglandin E1 (PGE1; alprostadil). PKA also modulates ion channels, including Kv7.5 channels, expressed in mouse CCSM cells [2]. More recently, exchange protein directly activated by cAMP (EPAC) has emerged as a distinct cAMP effector, inducing smooth muscle relaxation via Rap1/2 signalling, though its role in CCSM remains poorly defined.

Using selective activators of PKA (6-MB) and EPAC (007-AM), we investigated their contributions in mouse CCSM. EPAC-1 expression was confirmed at transcript (RT-PCR, qPCR) and protein levels (immunoreactivity in isolated CCSM cells), with antibody validation in mouse kidney cortex, which highly expresses EPAC-1 [3].

In isometric tension studies, CCSM pre-contracted with phenylephrine (PE; $3x10^{-7}$M) exhibited sustained phasic activity. EPAC activation (007-AM, $1x10^{-5}$M) produced modest but significant reductions in contraction frequency (6.87±1.47 min^{-1} to 5.17±1.07 min^{-1}; P=0.0156) and amplitude (0.99±0.16 mN to 0.86±0.16 mN; P=0.0276). Similarly, PKA activation (6-MB, $1x10^{-4}$M) reduced contraction frequency (6.70±0.59 min^{-1} to 4.57±0.47 min^{-1}; P=0.0001). However, co-activation abolished phasic contraction frequency (5.83±0.94 min^{-1} to 0.00±0.0 min^{-1}; P=0.0016) and amplitude (0.87±0.14 mN to 0.00±0.0 mN; P=0.0015).

At the cellular level, PE induced Ca^{2+} oscillations in isolated CCSM cells. 007-AM significantly reduced oscillation frequency (20.0±4.0 min^{-1} to 0.5±0.5 min^{-1}; P=0.0087) at high PE ($1x10^{-5}$M), previously identified as membrane potential-independent [2]. In contrast, 6-MB had no effect under these conditions (25.7±4.2 min^{-1} to 25.2±3.5 min^{-1}; P=0.8560) but abolished oscillations (9.5±0.9 min^{-1} to 0.0±0.0 min^{-1}; P=0.0445) at low PE ($1x10^{-7}$M), which are membrane potential-sensitive. This effect was prevented (8.0±1.0 min^{-1} to 15.5±2.1 min^{-1}; P=0.4896) by the Kv7 blocker XE-991, implicating Kv7 channels in PKA-mediated responses.

Collectively, these data show that EPAC-1 is expressed in CCSM and inhibits Ca^{2+} signalling, potentially via suppression of sarcoplasmic reticulum Ca^{2+} release, while PKA may promote relaxation through Kv7.5 channel activation and membrane hyperpolarisation. Although co-application of 007-AM and 6-MB abolished contractions, the ability of EPAC activation alone to suppress Ca^{2+} oscillations suggests a dominant role for EPAC rather than simple additive effects. Targeting cAMP effectors, particularly EPAC, alone or alongside PKA, may therefore offer a strategy to restore CCSM relaxation in patients unresponsive to PDE5Is.

C05

Engineering Patient-Derived Vascularised Cardiac Organoids to Target Mitophagy and Restore Cardiac Function in Type 2 Diabetes

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Introduction

Type 2 Diabetes Mellitus (T2DM) is known to affect over 5.6 million individuals in the UK alone (1) with cardiovascular disease (CVD) a common comorbidity. During T2DM-CVD, complex cellular dysfunction occurs involving endothelial cell loss, cardiac myocyte damage & subsequent fibrosis resulting in poor clinical outcomes (2, 3). Here we demonstrated for the first time the novel iPSC-derived vascularised cardiac organoid (VCO), derived from non-diabetic (ND) and diabetic (DB)-donors, demonstrating both cardiac and endothelial cells in a 3D structure, more closely emulating the human myocardium. Cardiac-endothelial cell mitochondria are susceptible to damage, leading to impaired function. Damaged mitochondria would be targeted for mitophagy, triggering the renewal of mitochondrial biogenesis, here we investigated the impairment of mitophagy within cells during T2DM. Telomere shortening and dysfunction is established in T2DM, with dysfunction of the telomeric repeat binding factor-interactive nuclear factor (TINF)-2 protein associated with mitochondrial loss in T2DM (4,5).

Aims and objectives

Data is currently lacking regarding the role of TINF2 signaling in cardiac or vascular cell signaling, cardiac toxicity and T2DM. Here we demonstrated the effects of TINF2 using the novel 3D VCO and its novel effects on cardiac and endothelial cell interaction in T2DM, and the re-establishment of mitophagy.

Methods & Results

iPSCs from DB and ND donors (3 individual donors, n3) were differentiated into 3D VCOs over a 20-day protocol, a novel approach combining mesodermal bodies programmed for either vascular or cardiac specific lineage commitment, allowing for the spontaneous formation of vascular-like networks directly within the cardiac tissue.

Both DB- and ND-derived iPSCs successfully differentiated into structurally mature VCOs, showing robust and consistent expression of key cardiac cellular markers and endothelial cellular markers confirmed with RT-PCR and western blot analysis (*n3, p<0.05-0.0001).*

Confocal imaging confirmed the presence of vascular-like networks within the VCOs, blood vessel-like structures closely integrated with surrounding cardiac cells. VCOs were successfully characterised for cellular markers using RT-PCR, western analysis & combined large scale RNA sequencing to demonstrate the T2DM profile, revealing significant changes in molecular signaling of AMPK, mTOR, PTEN, ANP & BNP (*n3, p<0.05-0.0001*).

Moreover, mitochondrial-targeted functional assays demonstrated T2DM-induced mitochondrial-associated depolarization, ROS increase and calcium-influx within the cardiac-endothelial cell population in ND and DB VCO, as well as impaired mitophagy and reduced lysosome clearance (*n3,*

p<0.05-0.0001). Correspondingly TINF2 expression was found to be significantly increased in DB VCOs compared to ND VCOs using RT-PCR, western blot analysis and ICC imaging (*n3, p<0.05-0.001*). Using shRNA, we transgenically knocked down (KD) TINF2 within DB VCOs, significantly reversing the previously demonstrated effects of DB, cardiac toxicity and mitochondrial loss of function. Mitophagy signaling was restored, whilst further reversing mitophagy and DB-associated loss of key signaling pathways (*n3, p<0.05-0.001*).

Conclusion

Using the VCO we demonstrate key functional loss of mitochondria and impairment of mitophagy within cardiac-specific tissue, in a glucose-independent state. We demonstrated the upregulation of TINF2 within cardiac-specific tissue for the first time, and as a key gene responsible for the impairment of mitophagy, setting the foundation for a novel therapeutic target for T2DM.

Ethics statement

All procedures were carried out in accordance with UK legislation.

C06

Nerve and agonist evoked contractions of mouse urethral smooth muscle critically rely on store operated calcium entry

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Introduction

Urethral smooth muscle (USM) contractions contribute to urethral closure pressure, and are modulated by agonists that liberate Ca^{2+} from intracellular stores via IP3 receptors (IP3Rs), such as phenylephrine (PE), or arginine vasopressin (AVP). Voltage dependent L-type Ca^{2+} channels (LTCC) contribute to USM contractility in numerous species, however a previous study in mice failed to detect any effect of LTCC inhibitors (nifedipine, nicardipine) on male USM contractions induced by PE (Drumm *et al.*, 2018). Conversely, inhibiting store-operated Ca^{2+} entry (SOCE) with Orai channel inhibitors markedly reduced PE responses. However, this study was limited in that only high PE concentrations (10 mM) were tested on USM responses and contractions evoked by nerve stimulation were not assessed.

Hypothesis/Aims

We hypothesised that contributions of LTCC may be apparent at lower agonist concentrations or during nerve stimulation, rationalizing exclusively high agonist doses would cause exaggerated Ca^{2+} release from internal stores and activate SOCE, masking possible contributions of LTCC to lower magnitude responses, thus importance of LTCC in mouse USM contractility under a range of experimental conditions may be underestimated. To test this, we carefully examined SOCE and LTCC contribution to murine USM contractile behaviours.

Results

qPCR revealed male and female USM expressed transcripts for LTCC (*Cacna1a,c,d,f*) and *Orai1-3* paralogs. *Orai1-3* expression was 4-5x greater in both sexes compared to LTCC (n=3). In male USM organ bath experiments, a range of PE concentrations (0.1-30 mM) or electrical field stimulation (EFS) at 1, 2, 5, 10 Hz (30 sec), led to dose-dependent and frequency dependent increases in contractile area and amplitude respectively. Nifedipine failed to affect PE or EFS evoked contractions at any agonist concentration (n=6) or EFS frequency (n=6). Sustained female USM contractions in response to 10 nM AVP were unaffected by nifedipine (n=5). FPL64176 (LTCC agonist) slightly increased EFS response amplitude by ~10% at frequencies >5Hz, and this was reversed by nifedipine (n=6). FPL64176 slightly increased (~10%) PE evoked contractile area of male USM at agonist concentrations of 0.3-30 mM (n=6), but did not affect AVP responses in female USM (n=5). All responses to PE, EFS and AVP were abolished by the IP3R antagonist 2-APB, highlighting dependence on sarcoplasmic reticulum (SR) Ca^{2+} release. Inhibition of SOCE with the Orai antagonist GSK 7975A (10 mM) significantly reduced PE induced contractile area of male USM (>50%, $P<0.001$) across agonist concentrations 0.1-30 mM (n=3). Amplitudes of EFS evoked contractions in male USM were significantly reduced (~50%) by GSK 7975A (n=13, P-0.002-<0.001), and Synta66 (Orai antagonist, 10 mM, P=0.008-<0.001, n=6). When EFS responses were reduced by either Orai antagonist, further addition of nifedipine caused a 5-20%

decrease in amplitude (n=13, P<0.001 (GSK), (n=6, P=0.001, Synta66). Both Orai inhibitors significantly reduced sustained contractions evoked by continuous application of 1 mM PE in males (P<0.01, n=5) or 10 nM AVP in females (P<0.0001, n=6), and subsequent application of nifedipine had no effect.

Conclusions

We conclude that in mouse USM, Ca^{2+} influx via SOCE through Orai channels, and not via LTCC, is a key Ca^{2+} source required to sustain agonist and nerve evoked contractions.

C07

Looking beyond "70Kg Male"; Including Female Simulated Participants in Ultrasound Teaching

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Ultrasound technology is rapidly becoming a popular tool in medical and basic science education allowing students to see working organs in real time, giving education which is rich in context and relevance. There is a problem, however, in the choice of subjects. To mitigate safeguarding concerns, particularly in classes which would involve exposure of chest and breast tissue, the conventional approach is to recruit "slim young males with good acoustic windows" as subjects for classes (Johnson et. al, 2025). Essentially thoracic ultrasound classes default to the "standard 70Kg (Physiologic) male". This practice has profound implications (Cheng & Yang, 2015). The subjects thus chosen are not representative of the diverse population that students will face in practice with profound downstream consequences. According to the British heart foundation, (British Heart Foundation, 2019) women are 50% more likely to receive an incorrect diagnosis during a heart attack, more likely to receive substandard treatment and more likely to receive inadequate aftercare leading to preventable cardiac deaths. Previous work by this group (Hegarty et al., 2025) surveyed female medical students who said that while 83% wanted to volunteer as subjects for thoracic ultrasound classes, only 14% were likely to do so. A popular solution proposed by those surveyed involved working with Simulated Participants (SP) as subjects in thoracic ultrasound classes. Including female SPs is not without its challenges as students may inadvertently cross professional/sexuality boundaries (Kearney et al., 2018).

This study explores female SP views about participating in an ultrasound chest exam involving breast exposure. After local ethics committee approval, female SPs completed a survey, which was used to purposively select participants for a focus group (n=4). Another focus group was conducted with SPs (n=3) who had been videoed undergoing a chest examination.

The survey was completed by 37% (40/108) of female SPs. Most were willing to participate in a thoracic ultrasound scan, either videoed or live. Their motivations for participating were to rectify the invisibility of women in the curriculum; enhance the clinical competency and confidence of medical students; de-sexualise women's breasts, especially among male students; and improve healthcare for women. Concerns they might have about participating related to the potential for male students, especially, to be unprofessional. Recommendations for preserving SPs' dignity included providing the students with a pre-brief about the scan and expectations about their behaviour and ensuring the appropriateness of the environment in which the scan occurs. Providing a separate pre-brief for SPs to outline the procedure and to assuage any concerns they might have was essential.

Including female SPs in chest examinations is a matter of patient safety in tackling avoidable cardiac deaths among women (British Heart Foundation, 2019) and for improving the confidence and

competency of students. This study offers guidance for promoting the dignity of female SPs in co-developing practical classes involving breast exposure.

C08

Understanding Myocardial Dysfunction and Heart Failure in Children with Single Ventricle Congenital Heart Disease

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Background: Paediatric heart failure is a severe disease with limited therapeutic options available. The predominant causes of paediatric heart failure are congenital structural malformations which cause progressive myocardial damage due to the abnormal physical forces associated with pumping blood. Some of the most severe congenital heart malformations are those where patients develop only a single ventricle and it is common for these patients to experience heart failure during childhood. A greater understanding of the causes and risk factors associated with paediatric heart failure is needed to develop novel precision therapies for improving myocardial function, which would greatly improve quality of life for these patients.

Methods: Blood samples were collected from patients with single ventricle congenital heart disease and clinical or subclinical heart failure to test for established and novel biomarkers of heart failure. Following identification of target pathways, further investigations into the implications of aberrant mechanical force and elevated oxidative stress were conducted in cardiomyocytes differentiated from induced-pluripotent stem cells (iPSC-CMs) and AC16 cardiomyocytes.

Results: Immunoassays of patient plasma revealed a significant correlation between elevation of plasma BNP levels and severity of heart failure, calculated by modified Ross score (R^2=0.233, $p > 0.0001$) . BNP is released by the myocardium under abnormal cardiac load, suggesting that altered mechanical homeostasis of the heart contributes to heart failure. Interestingly, we did not find the same trend with other biomarkers for heart failure, suggesting heart failure is driven by mechanical loading. Next, we manipulated cellular micromechanics in iPSC-CMs and found that stiffness regulates cardiomyocyte protein expression, function and contractility. To search for novel biomarkers, we conducted proteomic analysis of plasma. Of 569 proteins identified, 97 were up-regulated and 6 down-regulated compared to patient controls. Pathway enrichment analysis highlighted oxidative stress pathways in the up-regulated protein population. Aberrant mechanical load can lead to oxidative stress by production of reactive oxygen species (ROS), leading to heart failure. Similarly, elevated oxidative stress can disrupt calcium handling and cause contractile dysfunction. To explore this connection further, we next generated a single ventricle cardiomyocyte cell model by pharmacologically manipulating the activity of gap junction protein, Connexin43; a mechanosensitive protein that regulates oxidative stress, and which genetic mutations are linked to single ventricle congenital heart disease. We found that pharmacological modulation of Connexin43 hemichannel function regulates oxidative stress in cardiomyocytes, both basally and in response to H2O2-induced oxidative stress ($p > 0.05$).

Conclusions: Paediatric heart failure shares some common hallmarks with adult heart failure, however, mechanical load may play a more prominent role. High levels of oxidative stress experienced by single ventricle patients are likely due to abnormal mechanical loading of the heart, particularly when linked to pathogenic mutations in complexes that regulate mechanical and oxidative homeostasis, such as Connexin43. Our results suggest that these interactions between genetics, mechanical strain and oxidative stress drive myocardial dysfunction and heart failure. Identification of key disease mechanisms will support the development of new treatments and inform better long-term care for patients and their families.

C09

Beyond Engagement; Challenges to Staff and Students in Promoting Activism in Bioscience Education

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Sustainability has become a growing global concern reflected by the inception, in 2015, of the United Nations (UN) Sustainable Development Goals (SDG's) (United Nations, 2015). Consequently, the integration of these Goals (SDG's) into higher education has become a priority at local (Queen's University Belfast, 2024), national and international levels (Physiological Society, 2023). Our group has proposed a simple poster assessment as a means by which undergraduate students can engage meaningfully with the SDG's (McGahon et al., 2025). We found that the assessment fostered contextual learning, authentic assessment and increased awareness of the UN SDG's. An unexpected finding was that engagement with the assessment was deeply meaningful, with students reporting leaving the class with a sense of mission and activism. This work aims to explore this phenomenon, investigating student and academic perceptions of the potential of SDG education to promote, not just student knowledge and awareness of sustainability, but also engagement with Sustainable Education (SE) and how this may translate to activism.

After gaining ethics committee approval, a mixed-methods approach was used combining both electronic survey data and focus group discussions from 2nd Year Science (Human Biology, n=13) and Bioscience academics from various universities in the UK and Ireland (n=12). The survey assessed student and academic engagement with SE, as well as the importance they attach to it. Respondents were also asked whether such engagement was likely to lead to action and changes in sustainability behaviour.

Student data suggested that engagement with the SDG-related activities increased interest in sustainability issues, with 69% of students reporting that their interest increased "quite a bit" or "to a great extent" following the SDG-related assignment. This engagement also encouraged reflection on global challenges and personal values. Additionally, 85% of students reported they were "likely" or "very likely" to take future action and engage in activism, although action beyond the classroom was underreported. Educators generally recognised the need for SDGs integration into Biosciences education, with 11/12 rating its importance ≥ 4/5 (Likert Score) post engagement with teaching materials that linked SDGs to physiology. While many educators agreed that biosciences teaching should address global sustainability issues (12/12 "agree" or "strongly agree") and support student activism (9/12 ≥ 4/5 Likert Score), few reported actively promoting activism through their teaching (11/12 reporting ≤ 3/5 and only 1/12 reporting higher levels ≥ 4/5). Academics reported a range of pedagogical barriers and perceived risks preventing their promoting activism in their teaching.

These findings suggest a gap between recognising the importance of SE and actively promoting it within teaching practice. Educators were not aware of the directives from their organisation and discipline to

promote sustainability action. While academics may strive to enhance engagement and encourage reflection on SD, their concerns around their perceived role in addressing global challenges still outweigh their "partnership for the goals" and belief that they have a part in "activating" students.

C10

Circadian Regulation of Endothelial Adhesion Molecules in Diabetic Retinopathy

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Background: Endothelial cells (ECs) in the retinal vasculature act as essential gatekeepers, restricting inflammatory cell entry into the retina. In diabetes, circulating monocytes and granulocytes are elevated, driving chronic low-grade inflammation. This inflammatory milieu increases adhesion molecule expression on retinal ECs, promoting leukostasis and leukocyte infiltration into the retinal parenchyma—key contributors to the development and progression of diabetic retinopathy. Previous work has shown that ECs derived from diabetic patients display disrupted circadian rhythmicity and elevated ICAM-1 and VEGF expression, while patient fibroblasts exhibit altered temporal ICAM-1 expression. Our group has also demonstrated that leukocyte recruitment into the retina follows a time-of-day rhythm, suggesting that circadian regulation of adhesion molecules may be central to controlling inflammatory cell entry in diabetes.

Methods: Primary human retinal endothelial cells (HRECs) were exposed to stimuli mimicking the diabetic microenvironment: high glucose (25 mM D-glucose), inflammation (0.1 ng/ml TNF-α), oxidative stress (50 μM H_2O_2), or a combination of these factors. Adhesion molecule expression (ICAM-1, VCAM-1, P-selectin) was quantified using RT-PCR. To assess the role of the circadian clock, Bmal1 was silenced in HRECs, and changes in adhesion molecule expression were measured by RT-PCR and flow cytometry. Circadian rhythmicity was monitored using Per2-luciferase bioluminescence recordings to determine how diabetic-like stimuli alter endothelial clock function. Experiments were performed twice with n=3 technical replicates. Statistical analysis was done with GraphPad (2 Way ANOVA or cosinor for determination of circadian rhythmicity, $p<0.05$)

Results: High glucose or oxidative stress alone did not significantly increase ICAM-1 or VCAM-1 expression, whereas TNF-α robustly induced ICAM-1. Notably, combined treatment, reflecting the multifactorial nature of the diabetic microenvironment, produced a greater increase in adhesion molecule expression than any single stimulus. Silencing BMAL1 resulted in a marked reduction of ICAM-1 at both mRNA and protein levels, indicating that ICAM-1 expression is directly influenced by the endothelial circadian clock. Per2-luciferase recordings revealed that combination treatment disrupted circadian rhythmicity, inducing a phase shift in HREC molecular clock oscillations.

Conclusion: These findings demonstrate that the diabetic microenvironment synergistically enhances adhesion molecule expression in retinal endothelial cells and disrupts intrinsic circadian rhythms. The reduction of ICAM-1 following BMAL1 deletion highlights a direct regulatory role of the endothelial clock in controlling leukocyte adhesion pathways. Together, this work suggests that circadian dysregulation contributes to excessive leukocyte recruitment in diabetic retinopathy and may represent a novel therapeutic target for anti-inflammatory intervention.

C11

The role of sub-retinal pigment epithelium calcification in age-related macular degeneration

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Introduction:
Extracellular (ectopic) calcification is associated with age-related macular degeneration (AMD), the leading cause of irreversible vision loss in the Western world (1). A key pathological feature is the accumulation of drusen between the retinal pigment epithelium (RPE) and the Bruch's membrane, disrupting exchange of nutrients and oxygen, and the clearance of waste products (2). Drusen contain proteins, lipids, and calcium phosphate minerals, primarily hydroxyapatite (HAP) and, less frequently, whitlockite (WHT) (3–5). Although mineralisation is linked to the progression to end-stage AMD, the mechanisms involved in this process are not well understood.

Aims and Objectives:
This project aims to elucidate the intra- and extra-cellular factors that contribute to ectopic calcification in the sub-RPE space during the onset and progression of AMD.

Methods:
Human primary RPE cells (hRPE) were cultured on transwell inserts and treated with conditions that promote calcification. Long-term (LT, glycerophosphate, 2 weeks) or short-term (ST, high concentration sodium phosphate dibasic, 72 hours) calcification-inducing media, or pre-seeded synthetic calcium phosphate mineral were used. Transepithelial electrical resistance (TEER) was used to measure cell barrier functionality across multiple time points (n = 4). Gene expression changes were quantified using qPCR to examine markers associated with calcification and metabolism (n = 4).

Resipher assays and Seahorse measurements assessed RPE metabolic capacity measured as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). We compared values obtained from cells grown on HAP and WHT, with calcium diphosphate (CPD) and no-crystal used as controls (n = 6).

Data were analysed using t-test when comparing two groups, and one-way or two-way ANOVA when comparing three or more groups. Statistical significance was set at $p < 0.05$.

Results:
LT calcification inducing media induced a significant TEER decline over time compared with controls ($p < 0.05$), indicating progressive disruption of tight junction integrity. ST treatment similarly reduced TEER ($p < 0.05$), with the majority of TEER loss occurring within the first 24 hours ($p < 0.05$).

qPCR revealed that LT treatment significantly decreased expression of calcification- and glycolysis-related genes (ABCC6, HK2, PFKB3; $p < 0.05$), while ST treatment did not induce significant changes. This suggests LT treatment as a model for gene expression changes in sub-RPE calcification

Metabolic assays showed a significant metabolic shift towards glycolysis in crystal-treated hRPE, when compared to control cells at 4-week ($p < 0.05$) and 6-week ($p < 0.05$) differentiation. These results suggest impaired mitochondrial respiration and calcification-induced metabolic impairment.

Conclusions:

Here, we showed that extracellular calcification impacts RPE cell functionality, and metabolic capacity. These results indicate that calcification may directly influence retinal function, supporting clinical observations that link calcification to rapid progression toward end-stage AMD.

C12

Circadian rhythms of retinal microglia in early diabetes

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Title of the abstract: Circadian rhythms of retinal microglia in early diabetes

Authors - Varun Pathak, Jasenka Guduric-Fuchs, Hanagh Winter, Cara Crady, Beth Grazier, Tom Friedel, Eleni Beli

Study design: Preclinical study using control and STZ-induced diabetic CXCR1$^{gfp/+}$ reporter mice.

Purpose: This study investigated how diabetes affects the circadian and daily rhythms of microglial spatial distribution and morphology in the retina. Microglia are dynamic and exhibit circadian rhythms, but their retinal behaviour in health and disease is poorly understood.

Methods: Retinas from control and diabetic CX3CR1$^{gfp/+}$ mice were collected at different times within a 24hr Light/Dark and Dark/Dark cycle. Retinal flat mounts stained with collagenIV underwent microglial soma counts, ramification index, and vessel coverage analysis across layers using Imaris and ImageJ. Statistical significance and circadian rhythmicity were assessed using Two-Way ANOVA and Cosinor analysis ($p<0.05$).

Results: Under DD conditions, microglia displayed circadian rhythmicity with spatial and morphological changes over 24 hours. In controls, microglia numbers and ramification index peaked in the early morning and dropped at night by 45.1% and 53.8%, respectively. In early diabetes, these rhythms persisted, but soma numbers were reduced by 29.7% and ramification index dropped by 50%, with the deep plexus being mostly affected (54.3%). In LD conditions microglial numbers in controls peaked at midday and during night, while diabetic mice displayed altered rhythms. Furthermore, microglia were least active at night in diabetic mice, with light exaggerating circadian disruptions. Perivascular microglia were also quantified under LD conditions. Controls showed circadian rhythmicity, peaking during the day and dipping at night. Diabetic mice lacked rhythmicity, with perivascular microglia elevated by 43.1% compared to controls at midday and consistently high at all nighttime points.

Discussion: This study highlights significant circadian alterations in microglial activity under diabetic conditions. While rhythmicity persisted under constant darkness (DD), diabetes reduced microglial soma numbers and ramification index, suggesting heightened activation. Under LD cycles diabetic mice exhibited disrupted rhythms and elevated perivascular microglia particularly during the nighttime. These findings underscore heightened circadian dysregulation in diabetes and microglial sensitivity to light exposure.

Conclusions: Retinal microglial dynamics are regulated by an endogenous circadian clock, which remains functional in diabetes. However, light significantly influences microglial behaviour in diabetes, disrupting circadian rhythmicity and overriding normal dynamics.

C13

Senescence and circadian rhythms in Endothelial Colony Forming Cells

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Introduction

Among the hallmarks of aging is senescence, accompanied by chronic inflammation, and disruption of the circadian system. Senescent endothelial cells accumulate in blood vessels where they contribute to the development of cardiovascular diseases through chronic inflammation and vascular dysfunction.

Aims

The aim of this study was to investigate the relationship between senescence and circadian rhythms in Endothelial Colony Forming Cells (ECFCs) obtained from umbilical cord blood.

Methods

ECFCs were isolated from umbilical cord blood of full-term pregnancies with written consent from mothers and ethical approval REC 15/YH/0281. Senescence was induced by the treatment with Etoposide. Cells were treated with 100 nM Dexamethasone for circadian clock synchronisation followed by sample collection every 3 hours for two periods of two 24 hours cycles. Expression of circadian clock genes was examined by RT-qPCR. ECFCs were stably transduced with lentiviral luciferase reporter for *PER2,* subjected to etoposide induced senescence, and luminescence was monitored by a real time luminometer system. siRNA was used to silence BMAL1 *(ARNTL1)* gene and the expression of senescence and inflammatory markers was examined by RT-qPCR. Cosinor analysis and Two-way Anova (senescence, time, $P<0.05$) were performed using GraphPad Prism.

Results

Analysis of our previously published single cell sequencing data revelled differential expression of circadian genes *ARNT2, CRY1, PER1* and *RORA* between senescencet and young cells. Expression of clock genes including *ARNT1*, *PER2* and *CRY1* displayed phase shifts and changes in expression levels, compared to the young cells. Luminometer recording of PER2 expression revealed significant differences ($p<0.001$) between young and senescent ECFCs. On the other hand, knock down of BMAL1 in senescent cells led to upregulation of β-gal staining. Several interferon related genes were upregulated, including *ISG15* and *BST2*, but no differences were detected in the common senescence markers *CDKN1A* and SASP genes *IL8* and *IL6*.

Conclusion

We demonstrate an interplay between senescense and the circadian clock which could affect vascular function. Enhancing the clock may provide a strategy to delay senescence in vascular cells and preserve their function in ageing.

C14

Contribution of negatively charged residues in the tail of LINGO proteins to the shift in BK activation.

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Large-conductance Ca^{2+} and voltage-activated (BK) channels are key regulators of cellular excitability and are modulated by auxiliary subunits including β1-4[1,2], γ1-4[2] and LINGO1-3[3-5]. Co-expression of LINGO1-3 proteins with BK causes inactivation and shifts the voltage dependence of activation (V1/2ACT), towards more negative potentials[3,4] in BK:LINGO1 and BK:LINGO2, but to more positive potentials[5] in BK:LINGO3, compared to BK alone. Previous work has identified the intracellular C-terminal tail[6] as the most likely determinant of these effects and sequence alignment revealed a non-conserved residue in the proximal tail that differs between LINGO isoforms (E594 in LINGO1, D578 in LINGO2, S564 in LINGO3 and T569 in LINGO4), suggesting that this position may contribute to differences in V1/2ACT shifts.

To test this, BK and LINGO cDNA was transiently co-transfected into HEK293 cells and macroscopic currents were recorded at 37°C from inside-out patches using patch-clamp electrophysiology. Pipette and bath solutions contained 140mM K^+ and the [Ca^{2+}] at the cytosolic surface ranged from 100nM-10mM. Conductance-voltage (G-V) relationships were fitted with a Boltzmann function to determine V1/2ACT. Site-directed mutagenesis of D578 in LINGO2 and E594 in LINGO1 was examined in both full-length and inactivation-deficient (ΔMKMI) constructs[3]. Data is presented as mean±SEM, all experiments were carried out on five to eight patches and statistical comparisons were performed using one-way ANOVA.

In full length BK:LINGO1 channels, the V1/2ACT was 121 ± 2 mV in 100 nM Ca^{2+} as shown in previous studies[3,5]. Substitution of the equivalent residue from LINGO2 (BK:LINGO1E594D) resulted in a V1/2ACT of 129 ± 4 mV, (ns vs BK:LINGO1). In contrast, BK:LINGO1E594S significantly shifted V1/2ACT to 164 ± 4 mV in 100 nM Ca^{2+} ($p<0.01$ vs WT BK:LINGO1), consistent with the idea that swapping in the equivalent residue from LINGO3 at this position could predictably alter V1/2ACT.

Co-expression of BK:LINGO2 also produced a negative shift in V1/2ACT to 131 ± 2 mV in 100 nM Ca^{2+} as shown previously[4-6]. Swapping in the equivalent residue from LINGO1 (ie BK:LINGO2D578E) resulted in a V1/2ACT of 114 ± 2 mV, whereas swapping in the equivalent residue from LINGO3 (BK:LINGO2D578S, V1/2ACT=167 ± 2 mV) or LINGO4 (BK:LINGO2D578T V1/2ACT=157 ± 2 mV) produced significant positive shifts in V1/2ACT ($p<0.05$ vs WT BK:LINGO2).

When the LINGO2 inactivation particle was removed (BK:LINGO2ΔMKMI), the V1/2ACT in 100 nM was significantly shifted to 109 ± 3 mV ($p<0.01$ vs BK:LINGO2), suggesting that inactivation affected the accurate determination of V1/2ACT in full length BK:LINGO2. When the equivalent residue to LINGO1

was substituted into LINGO2 (BK:LINGO2D578EΔMKMI) the V1/2ACT was 96 ± 2 mV in 100 nM Ca^{2+} but this failed to reach statistical significance. In contrast, when the equivalent residue from LINGO3 was substituted in, to make BK:LINGO2D578SΔMKMI, the V1/2ACT predictably shifted positively to 152 ± 2 mV in 100 nM Ca^{2+} ($p<0.01$ vs BK:LINGO2ΔMKMI).

These findings suggest that a single conserved residue within the juxta-membrane region of the LINGO intracellular tail is a key determinant of BK channel V1/2ACT in both LINGO1 and LINGO2 since substitutions at this position produced predictable, isoform-specific shifts in V1/2ACT.

C15

Development of an ultrasound teaching resource: echocardiography in female subjects

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Echocardiography has been introduced recently into the first year Medicine course (cardiothoracic anatomy module) at Queen's University Belfast (QUB), where students gain hands-on experience of obtaining images from live subjects. Currently, this only involves male subjects, although we hope to include females soon. Meanwhile, to mitigate the lack of representation of female physiology in the curriculum, we produced a video resource of echocardiography on a female volunteer after consultation with currently practicing senior cardiac physiologists. This study aimed to assess efficacy and perceptions in 1st year medical students of the resource designed to demonstrate appropriate communication, consent, and professional conduct when conducting echocardiography in female patients, along with sex-based anatomical differences in cardiac anatomy.

Students for the 1st year medicine cardiothoracic anatomy module at QUB were invited to complete an anonymous pre- and post-resource viewing questionnaire, assessing understanding of informed consent, professionalism, patient empathy, communication, confidence in performing echocardiography on a female subject, and awareness of sex-related anatomical differences. Questions were in the form of Lickert scale responses and open-ended questions. Academic staff involved in teaching delivery were invited to review the resource through a focus group to determine whether intended learning objectives were met and to provide recommendations for further refinement. The work was approved by the Faculty of Health and Life Sciences Ethics Committee, QUB.

Twenty-nine students responded (21 female, 8 male) with a mean age 19.2 years (range 18-23 years). After viewing the resource, all students reported an increase in awareness of sex differences in cardiac anatomy (pre: 2.6±1.1 vs post: 4.5±0.5, Lickert score ± S.D.; $P<0.001$, Student's paired *t*-test), confidence in gaining informed consent (4.1±0.8 vs 4.5±0.5, $P<0.001$), comfort performing sensitive procedures (3.3±0.2 vs 4.7±0.5, $P<0.001$), and confidence identifying general cardiac structures (3.5±4.2 vs 4.2±0.5), $P<0.01$). Viewing the resource had no effect understanding patient dignity (4.8±0.8 vs 4.8±0.5). Other positive outcome included 100 % of students agreeing that the video increased awareness of empathy during intimate procedures, communication during intimate procedures, dignity when handling breast tissue and understanding patient dignity and comfort. Furthermore, all students felt the resource promoted professionalism and respect, 28/29 students agreed it helped with inclusive learning through female representation and all students felt it should be included in future teaching.

The staff focus group (4 members) felt unanimously the resource fulfilled its aims and provided several suggestions for improving the resource such as camera angles and additional images and points for narration.

We conclude that in the absence of hands-on experience for students of echocardiography with female subjects, a video resource is an effective way to convey anatomical differences between male and female cardiac anatomy along with sensitive and professional handling of breast tissue and considering patient comfort and dignity. It is an excellent means of increasing appreciation and understanding issues around intimate examinations in general. We found feedback from students and staff extremely constructive to allow the resource to evolve further, making a significant contribution to redressing the balance of female representation in this area of learning.