PL01

Enhancing student experience and graduate outcomes through inclusive physiology

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Across the United Kingdom, many level two and three learners are from regions with high social deprivation and poor progression to higher education. It is widely acknowledged that outreach activities can address this as they seek to ensure secondary school students do not make choices that limit access to university thence science-based careers simply because they do not know they exist. At The University of Salford, related initiatives such as the “Salford Schools Network” now mean 40 % of students are from low-income backgrounds and a significant proportion are from areas with the lowest progression to higher education *nationally* [1].

However, facilitating progression to higher education is only the first step towards improving inclusivity and graduate outcomes for all. The career achievement gap experienced by university students from disadvantaged or underrepresented backgrounds is well known and a barrier to employability or progression to PhD. Multiple and complex socioeconomic factors underpin this. Yet, it remains the case that while at university many disadvantaged students are - *for the first time* - made aware of thus develop ambitions to enter physiology-based research and related careers. However, this late realisation means they are often behind the curve in terms of engagement with extracurricular activities that broaden horizons and enhance the CV. In many cases this arises from a lack of opportunity rather than disengagement which is especially true if students come from communities with limited awareness of academia and related careers, or they are the first in their family to go to university. Despite being every bit as *academically* capable as students from more advantaged backgrounds, they do not have the same level of experience and insight. This frequently means they are less competitive at interview.

In my lecture I will cover the various extra-curricular initiatives I have put in place to level up disadvantaged students at The University of Salford. Examples include development of a career hub, launch of Salford’s Research Career Working Group, facilitation of undergraduate engagement with research and The Physiological Society and the introduction of international mobility opportunities. Fundamentally, all seek to enhance widening participation in physiology-based and wider research thus provide the experience and insight that is often lacking but essential for progression to related careers.

I also hope to demonstrate that these initiatives are transformative. Resulting impact is not metric-based but tangible; the day a disadvantaged student holds their own at a scientific meeting or is offered a PhD position, a place at medical school or achieves any other career ambition. The added value is considerable, especially given that three years prior, most were completely unaware such career pathways existed.

[1]. Namvar S et al. (2019). Employability: breaking the mould, 81-87

PL02

Biophysical and molecular mechanisms of voltage-gated sodium channel gating: A quarter-century of resurgent sodium current

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Electrical signaling in most nervous systems depends upon sodium current, which flows through voltage-gated sodium channels. From their first voltage clamp measurements, Hodgkin and Huxley (1952) recognized the “dual effect” of voltage on the sodium conductance: In modern terms, depolarization first activates and then inactivates voltage-gated sodium channels, such that current flows only briefly at positive potentials and requires a recovery period at negative potentials before it can flow again upon subsequent depolarization. Both the voltage-dependence and time course of recovery from inactivation set the refractory period for action potential firing. Although tetrodotoxin-sensitive voltage-gated sodium currents show little heterogeneity across neurons, about 25 years ago, we found that cerebellar Purkinje neurons show a qualitatively distinct form of sodium channel gating. There, voltage-gated channels open briefly upon depolarization, permitting transient sodium current to flow, but the same channels reopen to pass a “resurgent” sodium current upon repolarization, indicative of a less stable form of inactivation. This component of sodium current is present in many other neurons typified by rapid or burst firing of action potentials. Changes in resurgent sodium current have been predicted to occur in disorders of excitability, e.g., in association with paroxysmal extreme pain disorder, epilepsy, paramyotonia congenita, long-QT syndrome, and neuropathy. A primary question relevant to the understanding of sodium channel gating, as well as the action potentials that result, is what the mechanisms of resurgent current are, both biophysically and molecularly. In early work, we proposed that sodium channels that generate resurgent current are subject to a rapid, voltage-dependent, open-channel block by an endogenous blocking particle. With depolarization, channels would open and, instead of inactivating in the usual manner, would rapidly become blocked by the native blocker. With repolarization, the blocker would unbind, briefly leaving the pore open to pass resurgent current before channels inactivated normally (at moderately negative potentials) or deactivated (at more negative potentials). Since the time that this mechanism was proposed, many electrophysiological as well as structural results have emerged, which have not only rendered this hypothesis more precise, but also linked it more clearly to research that preceded it. In this talk, I will place the idea of open-channel block as a mechanism for resurgent sodium current in the context of earlier and later studies of ion channel biology and discuss the implications for neural signaling, pathophysiology and drug targeting.

PL03

Kings and Queens of the mountains: human physiology at high altitude

Andrew Murray

undefined

As we ascend to high altitude, air pressure falls and our bodies experience low oxygen availability - a condition known as hypoxia. In response, our heart rate and breathing rate increase - an attempt to maintain the supply of oxygen to our vital organs. Over time, levels of oxygen-carrying red cells increase in our blood. Meanwhile, the cells of our bodies, and the oxygen-consuming mitochondria within, re-wire their metabolism. This serves to decrease our bodies' demand for oxygen and improve the efficiency at which we use this increasingly scarce but vital resource. Despite this process of acclimatisation, we remain limited by the low oxygen available to us, and this impacts our capacity to function, limiting our ability to exercise and think. Pregnancy at altitude poses a particular challenge, restricting growth of the developing fetus and potentially endangering the health of both mother and her offspring. In human populations that have spent thousands of years at altitude, including groups resident in the Himalayas and the Andes, there has been a selection of physiological traits, underpinned by genetic differences, which enable people to live, work and successfully reproduce. In this lecture, we will look at the responses of our bodies to altitude, and consider the different evolutionary strategies adopted by high altitude dwelling people. We will look at adaptations that support pregnancy at high altitude and will see how research into physiology at altitude is helping us to understand the condition of patients at sea level who experience hypoxia in common, but life-threatening contexts, such as complications of pregnancy or critical illness.

PL04

“The Place of Physiology in the Neuroscience of Memory”

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Historically, several biomedical disciplines have played a part in developing our understanding of memory, with physiology playing a key role. Best known is the discovery of long-term potentiation (LTP) in the hippocampus by Terje Lømo in Norway (Lømo, *Acta Physiologica Scand.* 1966) and the first full report of the phenomenon by Bliss and Lømo (*Journal of Physiology*1973). From the outset, LTP was found to have properties desirable of a memory mechanism – that it long outlasts the duration of the initiating stimulus, is pathway specific, and apparently associative in character. Another key discovery was that of place cells in the hippocampus by O’Keefe and Dostrovsky (*Brain Research* 1971), a finding followed in the years afterwards by those of other spatially tuned cells such as head-direction units (Taube et al, *J. Neuroscience* 1990) and grid cells (Hafting et al,*Nature* 2005). Collectively, these helped build the idea of the hippocampus being key to spatial memory. An entirely different learning system, based in the striatum, is instrumental in the learning of actions and habits as established in physiological, human functional imaging data and computational models; it deploys an error-correcting learning rule (Schultz et al, *J Neuroscience* 1992; Montague et al, *J Neuroscience* 1996).

It would, however, be wrong to suppose that the significance of these findings rests solely on physiological data. Neuroanatomy has also played a key role, dating back to Cajal’s Croonian Lecture to the Royal Society in 1894; likewise neuropsychology, as in Hebb’s conjectures about cell-assemblies in the brain and his proposal for a simple synaptic learning rule, as exemplified by LTP (Hebb, *The Organisation of Behavior,* 1949); pharmacology also weighed in with the discovery that glutamate is the major excitatory transmitter of the brain and that selective glutamate antagonists such as D-AP5 blocks the induction of LTP without affecting baseline glutamatergic transmission (Collingridge et al, *Journal of Physiology,* 1983). Behavioral studies have also contributed by rigorously testing the idea that activity-dependent synaptic transmission is necessary for the formation of episodic and spatial memory traces (Morris et a, *Nature,* 1986).

Contemporary studies using several of the remarkable technological innovations of recent years (e.g. optogenetics, calcium imaging in awake animals) are building on these foundations in intriguing ways. This lecture, with its requested historical backbone, aims to outline progress over the years and the challenges that remain in understanding memory as a fundamental feature of higher cognitive function.

PL05

Store-operated calcium channels: from nano domains to in vivo pathophysiology

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Communication between and within cells is essential for the development and survival of any complex organism. Cells converse with each other through the judicious use of a complement of chemical messengers, including neurotransmitters, hormones, and paracrine factors. These molecules bombard the cell surface, generating further signals, or second messengers, within the cell that then trigger the appropriate responses. Although several hundred hormones, neurotransmitters, and other molecules can stimulate cells, the number of intracellular second messengers they activate is remarkably small. Perhaps the most widespread and versatile of these second messengers is the calcium ion (Ca^{2+}).

Store-operated Ca^{2+} channels are a universal way to raise cytosolic Ca^{2+} in eukaryotic cells. These channels are particularly important in electrically non-excitable cells and are indispensable for immune cell function.

Growing evidence shows that store-operated channels engage in private conversations with downstream targets, through the use of spatially restricted Ca^{2+} signals, called Ca^{2+} nanodomains, which build up rapidly near open channels. Scaffolding proteins juxtapose with store-operated channels and position Ca^{2+}-dependent signalling molecules within the nanodomain, forming a signalosome. One such signalosome, involving AKAP79, allows for local Ca^{2+} signals to activate transcription factors of the NFAT family which then regulate gene expression. In this talk, I will describe properties of store-operated channels, how they participate in a membrane-delimited signalling complex to activate nuclear gene expression and how targeting the signalosome might open up new approaches for treating human disease.

PL06

Neuropeptide-Y: being “unsympathetic” to the broken hearted

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William Bayliss and Ernest Starling are famous as pioneers in cardiovascular physiology but are also responsible the discovery of the first hormone (from the Greek “setting in motion”), the intestinal signalling molecule and neuropeptide secretin in 1902[1]. My research group focuses on neuropeptides and neuromodulators that influence cardiovascular autonomic control as potential biomarkers in disease and tractable targets for therapeutic intervention. Acute myocardial infarction (AMI) and chronic systolic heart failure (CHF) result in high levels of cardiac sympathetic stimulation, which is a poor prognostic indicator. Whilst beta-blockers improve mortality in these conditions by preventing the action of the neurotransmitter noradrenaline, a substantial residual risk remains. Recently, we have identified the sympathetic co-transmitter neuropeptide-Y (NPY) as being released during AMI, leading to larger infarcts[2] and life-threatening arrhythmia[3] in both animal models and patients. Moreover, in patients with severe CHF, local cardiac NPY levels correlate with mortality[4]. I will present recently published and unpublished data demonstrating that peripheral venous NPY levels are associated with heart failure hospitalisation and mortality after AMI[5], and all cause and cardiovascular mortality in CHF, even when adjusting for known risk factors (including BNP). We have investigated NPY expression in human and rat stellate ganglion and cardiac tissue and used human induced pluripotent stem cell (hiPSC) cardiomyocytes to manipulate NPY neurochemistry using state-of-the-art imaging techniques, establishing the receptor pathways responsible for NPY signalling. We propose NPY as a new mechanistic biomarker in AMI and CHF patients and aim to determine whether specific NPY receptor blockers can attenuate the development of heart failure.

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SA01

A day-night rhythm in the heart including the sinoatrial node: an intrinsic mechanism and neurohumoral regulation

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In mammals, there is well known to be a day-night rhythm in the electrical activity of the heart (in heart rate, PR interval, QRS duration and QT interval) and arrhythmogenesis[1]. This has previously been attributed to short-term regulation of ionic conductances in the heart by the autonomic nervous system, but this explanation has been challenged[1]. Instead, recent studies of the mouse have provided an alternative explanation.

In the sinoatrial node, we have shown that there is an intrinsic mechanism that can explain or at least contribute to the day-night rhythm in heart rate: there is a day-night rhythm in ion channels, including the pacemaker channel HCN4, and block of HCN4 abolishes the day-night rhythm in heart rate *in vivo* and *in vitro*[2]. We have shown that there is a functioning circadian clock in the sinoatrial node that could be driving the day-night rhythm in ion channel gene transcription: in the case of HCN4 at least, knockout of the clock gene *Bmal1* abolishes the day-night rhythm in *Hcn4*[2]. However, data are emerging for a system of transcriptional 'combinatorial regulation' in which a specific combination of transcription factors is obligatory for gene transcription: there is a day-night rhythm in the sympathetic nervous system and chronic b-adrenergic receptor blockade also abolishes the day-night rhythm in ion channel transcripts (including *Hcn4*)[3]. For the atrioventricular node, a similar picture is emerging: there is day-night rhythm in ion channel transcripts and a functional circadian clock[4] and genetic knockout of *Bmal1* blunts the day-night rhythm in the PR interval (unpublished data). Again the picture is similar for the ventricles with a day-night rhythm in ion channel transcripts and a functional circadian clock[3]; the day-night rhythm in ion channel transcripts is suggested to be responsible for the well-known vulnerability to ventricular tachyarrhythmias at the start of the awake period. Once again there is evidence of combinatorial regulation: genetic knockout of *Bmal1* abolishes the vulnerability to ventricular tachyarrhythmias at the start of the awake period[5]; chronic b-adrenergic receptor blockade abolishes the day-night rhythm in ion channel transcripts[3]; and RU486 (an antagonist to the glucocorticoid receptor, Nr3c1; of interest because there is a day-night rhythm in plasma corticosteroid) again abolishes the day-night rhythm in ion channel transcripts as well as the vulnerability to ventricular tachyarrhythmias at the start of the awake period (unpublished data). ATAC-seq has shown a day-night rhythm in accessibility to certain genes (chromatin has to be made accessible for transcription to take place) and in many cases of genes showing a day-night rhythm in accessibility there is a consensus binding site for Nr3c1 (a transcription factor as well as a receptor) (unpublished data). Genetic knockout of Nr3c1 also abolishes the

vulnerability to ventricular tachyarrhythmias at the start of the awake period (unpublished data). In summary, a new explanation of the day-night rhythm in the heart is beginning to emerge involving a summation of inputs from an intrinsic cardiac circadian clock and neurohumoral factors.

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SA02

Inflammatory Remarks: Targeting pro-inflammatory Galectin-3 prevents cardiac conduction system dysfunction in heart failure

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Background: In patients with heart failure (HF), concomitant cardiac conduction system (CCS) dysfunction is an important predictor of mortality. Despite this, the molecular mechanisms underlying HF-induced CCS disease are poorly understood. Inflammation is a hallmark and mechanistic proponent of ventricular remodelling in chronic HF but its involvement in CCS dysfunction is presently unknown.

Methods and Results: We assessed the global signature of HF-induced molecular remodelling of the sinoatrial (SAN) and atrioventricular node (AVN) in the mouse transverse aortic constriction model of HF. Transcriptomic analysis using RNAseq and mass spectrometry-based proteomics combined with single nucleus RNAseq data intersection determined that downregulated proteins were predominantly enriched for ion channels involved in pacemaking, whereas upregulated proteins annotated to the immune-inflammatory response. In particular, striking enrichment of the macrophage population was observed in the failing CCS alongside a significant increase in expression of the macrophage-secreted proinflammatory protein Galectin-3 (Gal-3), a biotarget and biomarker in human HF.

To investigate a functional role for Gal-3 in HF-induced CCS remodelling, sham-operated and HF animals were randomised into anti-Gal-3 treated and untreated groups. Animals in the anti-Gal-3 treated group received 100 mg/kg/day modified citrus pectin (MCP), a well-characterised and clinically utilized Gal-3 inhibitor, starting from the day of surgery and continuing for 8 weeks. At termination, the impact of Gal-3 inhibition on CCS electrophysiological parameters was tested *in vivo* and in Langendorff-perfused hearts: MCP treatment significantly blunted prolongation of sinus cycle length, corrected SAN recovery time and the rate-corrected PR interval seen in untreated HF animals, whereas the Wenckebach cycle length and AVN effective refractory period were unaffected. To further evaluate SAN remodelling, high resolution unipolar multielectrode array mapping was carried out on the endocardial surface of isolated SAN preparations from the four groups of animals. Analysis of activation maps demonstrated that HF SAN had an inferior leading pacemaker site as well as slower conduction than control animals, changes that were restored to control levels in the MCP treated TAC group. Unipolar fractionated electrograms - indicative of structural and electrical remodelling resulting in asynchronous activation of myocytes - were significantly more prevalent in untreated HF animals, and the incidence of complex fractionated electrograms were also restored to control levels in the HF group receiving MCP treatment. Finally, using sharp microelectrodes, intracellular action potentials were recorded from the compact AVN. Strikingly, MCP treatment abrogated the HF-induced reduction in the resting membrane potential, upstroke velocity, action potential amplitude and slope of diastolic depolarisation.

Conclusions: These data provide novel proof-of-concept that Gal-3 inhibition prevents CCS dysfunction in HF of a pressure overload pathophysiology. Studies incorporating precision transgenics to study the impact of CCS-specific inflammation, coupled with state-of-the-art imaging mass cytometry to characterise the precise Gal-3 secreting macrophage population infiltrating the failing human and mouse CCS are underway.

SA03

The switch from nonfiring to firing mode in cells of the sinoatrial node.

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The sinoatrial node in the heart is composed of pacemaker cells which generate the heartbeat. Individual pacemaker cells exhibit substantial heterogeneity in their electrophysiological properties. Recently, we discovered that sinoatrial node cells can switch abruptly between a firing mode, in which they regularly fire action potentials, and a nonfiring mode, in which they stop firing for a certain period of time. Within the sinoatrial node network, firing and nonfiring cells interact electrically via gap junctions. Nonfiring cells slow action potential frequency in cells with intrinsic automaticity and, conversely, firing cells recruit nonfiring cells to fire. This mechanism is termed tonic entrainment and is important for the ability of the leading pacemaker region to generate regular electrical discharges that control electrical activation of the entire heart. Most importantly, this mechanism can be tuned by the autonomic nervous system. We show that the proportion of firing cells can be increased by the sympathetic nervous system via cAMP-dependent regulation of the pacemaker ion channel HCN4, thereby stabilizing sinus node function. Lack of cAMP regulation of HCN4 in a genetic mouse model results in inappropriately increased SAN heart rate responses to vagal nerve activity in vivo, sinus bradycardia, dysrhythmia and chronotropic incompetence.

SA04

Transcriptomic responses to disuse muscle atrophy and exercise-induced muscle hypertrophy

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Background: Skeletal muscle atrophy is a prominent characteristic of many disease states, however, the extent of similarities and/or differences in the underpinning mechanisms between atrophying conditions in unclear. Two of the most prevalent and costly atrophic conditions are ageing and disuse, with resistance exercise training (RET) the most effective nonpharmacological countermeasure. We conducted gene-level and network-level meta-analyses to compare transcriptomic signatures of disuse and RET, plus young and older RET to establish the molecular features of, and therapeutic targets against, muscle atrophy in conditions of high socio-economic relevance.

Methods: Integrated gene- and network-level meta-analysis was performed on publicly available microarray data sets generated from young (18–35 years) *m. vastus lateralis* muscle subjected to disuse (unilateral limb immobilization or bed rest) lasting ≥7 days or RET lasting ≥3 weeks, and from older (≥60 years) *m. vastus lateralis* muscle subjected to RET (≥3 weeks).

Results: Disuse and RET displayed predominantly distinct transcriptional responses, and transcripts altered across conditions were mostly unidirectional. However, disuse and RET induced directly inverted expression profiles for mitochondrial function and translation regulation genes, with COX4I1, ENDOG, GOT2, MRPL12, and NDUFV2, the central hub components of altered mitochondrial networks, and ZMYND11, a hub gene of altered translation regulation. A substantial number of genes (*n*=140) up-regulated post-RET in younger muscle were not similarly up-regulated in older muscle, with young muscle displaying a more pronounced extracellular matrix (ECM) and immune/inflammatory gene expression response. Both young and older muscle exhibited similar RET-induced ubiquitination/RNA processing gene signatures with associated PWP1, PSMB1, and RAF1 hub genes.

Conclusions: Transcriptional signatures of disuse are not simply the converse of RET, with limited opposing gene profiles. Therefore, the mechanisms of atrophy cannot be derived from studying hypertrophy alone. Moreover, this provides a molecular basis for understanding why RET fails to target all transcriptional features of disuse. Loss of RET-induced ECM mechanotransduction and inflammatory profiles might also contribute to suboptimal ageing muscle adaptations to RET. Disuse and age-dependent molecular candidates further establish a framework for understanding and treating disuse/ageing atrophy.

SA06

Boosting nitric oxide bioavailability as a strategy for enhancing neurovascular coupling and preventing cognitive dysfunction

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The brain´s function and structural integrity rely on a tightly regulated delivery of metabolic substrates (glucose and oxygen) matching the ongoing neuronal activity. This process – neurovascular coupling (NVC) – is critically orchestrated by nitric oxide (•NO) via the glutamate NMDAr-nNOS-sGC pathway, especially in the hippocampus, a brain region involved in memory processing (Lourenço et al., 2014, Figure 1). The dysfunction of NVC, linked to compromised •NO bioavailability and bioactivity, has been increasingly associated with neuronal dysfunction in several neurodegenerative conditions, such as Alzheimer's Disease and Vascular Cognitive Impairment and Dementia (VCID), being recognized as a relevant contributor to dysfunctional cascade leading to neurodegeneration and cognitive decline. In addition to the canonical enzymatic pathways, •NO can be produced upon the sequential reduction *in vivo* along the nitrate-nitrite-NO pathway. In this line, we hypothesized that dietary nitrate can be used as a strategy to foster •NO-dependent NVC under conditions of limited •NO bioavailability.

We tested our hypothesis in two rodent models mimicking specific features of VCID: 1) 2VO rats modeling cerebral hypoperfusion and 2) diabetic Goto-Kakizaki (GK) rats modeling microvascular dysfunction. Dietary nitrate intervention was achieved by providing sodium nitrate in water *ad libitum* for 8-12 weeks. The NVC functionality was accessed by measuring hemodynamic responses to glutamatergic activation in the hippocampus *in vivo* by laser Doppler flowmetry simultaneously with coupled •NO dynamics by electrochemical methods. The spatial working and reference memory dependent on hippocampal function was assessed in the Barnes maze paradigm. NADPH oxidase-mediated superoxide formation was detected by a lucigenin-dependent chemiluminescence assay. All the procedures were performed in compliance with the ethical regulations for animal-based research.

We found a compromised NVC in response to glutamatergic activation in both animal models (CBF changes were reduced to 41±3% in 2VO and 33±4% in GK as compared to their controls), which in GK rats were coupled with •NO transients with a shorter and faster profile. Of notice, in both GK and 2VO rats, these findings were coupled to a compromised spatial memory performance. As hypothesized, the intervention with dietary nitrate was able to counteract the spatial memory decline in both animal models which was correlated with an improvement in the NVC. Also, dietary nitrate reduced the NADPH activity in the hippocampus of both 2VO and GK rats.

Overall data support a close mechanistic association between hippocampal neuronal-triggered •NO concentration dynamics, hemodynamic responses, and cognitive performance, establishing the functionality of NVC as a critical early factor to consider in the cascade of events leading to cognitive decline in VCID that can be improved by dietary nitrate intervention.

Neurovascular coupling in hippocampus is mediated via diffusion by neuronal-derived nitric oxide. Lourenço CF, Santos RM, Barbosa RM, Cadenas E, Radi R, Laranjinha J.Free Radic Biol Med. 2014 Aug;73:421-9

SA07

Sympathetic neural responses and adaptation to the challenge of exercise, and to high altitude stress

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Sympathetic nerves play a pivotal role in control of systemic vascular resistance and autonomic regulation of arterial blood pressure. Furthermore, microneurography, the technique for recording neural activity directly from peripheral nerves innervating skeletal muscle vessels, is a fundamental tool for studying sympathetic modulation of vascular resistance and blood pressure during a variety of physiological and environmental challenges. During a bout of vigorous exercise, increased muscle sympathetic nerve activity (MSNA) and ensuing vasoconstriction in contracting muscle avert a drop in arterial blood pressure and vital perfusion when metabolic vasodilation would otherwise threaten to outstrip the pumping capacity of the heart. Notably, increased sympathetic outflow to skeletal muscle vasculature during exercise arises from integration of multiple neural inputs to the lower brainstem, including central command, afferent feedback from contracting muscle, and the arterial baroreceptors. In healthy individuals, it is apparent that exercise training can lead to heightened basal MSNA and resetting of the vascular sympathetic baroreflex, adjustments that might be important for maintaining arterial blood pressure in the face of cardiac and vascular adaptation induced by years of athletic training. Exposure to high altitude (HA) hypoxia is another physiological state in which skeletal muscle vasodilation challenges sympathetic modulation of vascular resistance and arterial blood pressure. Although relatively few in number, microneurographic studies indicate that heightened MSNA is a feature of HA exposure, not only in lowland natives, but also in highland populations who have generational exposure to ambient hypoxia. This invited talk will explore mechanisms underpinning the sympathetic neural adaptive responses to exercise in health and compare these with alterations in sympathetic outflow that are a feature of altitude acclimatization and adaptation.

SA08

Altered blood pressure regulation during simulated orthostatic stress in exercise trained premenopausal women with functional hypothalamic amenorrhea

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In premenopausal women, exercise training is commonly associated with menstrual disturbances, including functional hypothalamic amenorrhea (FHA). FHA is characterised by chronic estrogen deficiency similar to that observed in postmenopausal women. Despite being young and otherwise healthy, exercise trained women with FHA (ExFHA) demonstrate impaired endothelial function, increased regional vascular resistance, and decreased regional blood flow. Estrogen deficiency is thought to play an important role. Accordingly, similar findings have been reported in postmenopausal women. However, in contrast to postmenopausal women, ExFHA women demonstrate low, rather than elevated, resting arterial blood pressure. In postmenopausal women, estrogen deficiency due to menopause is assosciated with both increased sympathetic nervous system activity and augmented activation of the renin-angiotensin system. Our investigations of blood pressure regulation in young premenopausal women with ExFHA, compared with age- fitness- and body mass-matched eumenorrheic women, identified augmented lower limb skeletal muscle sympathetic nerve activity (MSNA) yet lower arterial blood pressure during simulated orthostatic stress using lower body negative pressure (LBNP). Further, in ExFHA, non-activation of the renin-angiotensin system despite increasing LBNP (0, 10, 20 and 40 mmHg) was also observed. Thus, otherwise healthy ExFHA women demonstrate low arterial blood pressure and disruption of the normal circulatory response to an orthostatic challenge: namely plasma renin, angiotensin II and aldosterone fail to increase and blood pressure is defended by augmented sympathetic vasoconstrictor responses. This invited talk will examine what is known about the uncoupling of the reflex sympatho-neural and renin-angiotensin system responses to a hypotensive stimulus in estrogen deficient physically active premenopausal women with FHA.

SA09

Blood pressure control during exercise: implications for hypertension

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An exaggerated blood pressure (BP) response to maximal exercise is an independent risk factor for cardiovascular events and mortality. People with hypertension have an elevated muscle sympathetic nerve activity (MSNA) at rest and during exercise, which is in part mediated by the metaboreflex. In people with hypertension, it was unclear if treating resting BP to guideline levels could reduce the activity of the metaboreflex and normalise the rise in BP during exercise. In our studies it was found that individuals with treated and controlled, treated and uncontrolled, and untreated hypertension have an exaggerated BP response to incremental exercise testing ($\dot{V}O_2$ peak testing) and metaboreflex isolation compared to age matched healthy controls. Heightened metaboreflex sensitivity in these individuals, could in part, be due to impaired functional sympatholysis during exercise. Dietary nitrate intervention lowers resting BP in hypertensive individuals, whilst also improving exercise performance, blood flow and exercise BP in healthy individuals. In our study, despite increased levels of plasma nitrates and nitrites in patients with treated-controlled hypertension, 4 weeks of dietary nitrate supplementation had no impact on the submaximal or maximal BP response to $\dot{V}O_2$ peak testing or metaboreflex isolation compared to a placebo. This invited talk will examine what is known about the abnormal metaboreflex during exercise in hypertension, potential treatments, and also future directions.

SA10

Abnormal reflex rise in sympathetic activity during exercise in heart failure and the impact of exercise training

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Patients with heart failure with reduced ejection fraction (HFrEF) are characterised by increased sympathetic nerve traffic directed to skeletal muscle and exercise intolerance; both associate with increased mortality. Our studies with direct microneurographic recordings of muscle sympathetic nerve activity (MSNA) revealed a qualitative difference in MSNA response during mild exercise in HFrEF patients compared with age-matched healthy controls: an increase in MSNA in patients vs. a drop in healthy controls. The elevation in MSNA at rest and during exercise in HFrEF relates inversely to peak oxygen uptake, supporting a neurogenic limit to exercise. The augmented exercise-induced sympathetic response is due partially to greater muscle metaboreflex activation and is exaggerated in those with low exercise capacity. When patients undergo 6 months of exercise training, MSNA burst frequency is lowered, peak oxygen uptake is improved and the autonomic benefit is particularly effective in those who can train at higher intensity. This sympathoinhibitory effect of training partially reflects a blunted muscle metaboreflex but little is known about the contributions of other reflexes. This invited talk will examine what is known about the abnormal reflex rise in sympathetic activity during exercise in HFrEF patients and the relative contributions of excitatory and inhibitory reflexes which may be modifiable by exercise training.

SA11

Viroporins: structure, function and potential as antiviral targets

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Ion channels are targeted by ~20% of currently licensed drugs, yet those encoded by viruses, “viroporins” are neglected by comparison. This is despite the clinical precedent of adamantane drugs targeting the influenza A virus (IAV) M2 proton channel. Viroporins play essential roles during the lifecycles of many pathogenic viruses yet, with few exceptions, precise understanding of their function within virion and/or host cell membranes remains limited. One major bottleneck has been limited usefulness of prototypic small molecule inhibitors.

Viroporins also perform non-channel related functions, confounding mutagenesis studies. Thus, we have focused upon improving small molecules as tools to investigate their properties. Druggable binding sites identified by prototypic drugs can be refined via an array of approaches yielding inhibitors with improved fidelity and utility. Iterative increases in both structural and functional understanding of viroporins can identify new biological roles, simultaneously forming a platform for future therapeutic discovery.

SA12

Role and purpose of microbial rhodopsins in giant viruses.

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Microbial rhodopsins are a family of light-sensitive membrane proteins that perform various functions upon light illumination. Recently, rhodopsin-like genes were found in genomes of nucleoplasmic large DNA viruses, which opened a discussion about the potential biological implications of the light-sensitive machinery of giant viruses. The viral rhodopsins family comprises two subgroups, namely group 1 (VirR1) and group 2 (VirR2), that differ phylogenetically from non-viral rhodopsins (Yutin and Koonin 2012). We studied members from both groups to shed light on their molecular and cellular functions. Using the patch-clamp method, we tested the electrophysiological properties of VirR-expressing human neuroblastoma cells (SH-SY5Y) in both light and dark conditions. We observed that viral rhodopsins expressed well, but showed strong retention in the cytosol. To address the plasma membrane localization issue, we tested multiple N- and C- terminal modifications of VirR constructs. When we supplemented one of the VirR1 proteins, VirChR1 with p2A self-cleavage peptide prior to fluorescence tag, we were able to get measurable photocurrents. The electrophysiological characterization revealed that VirChR1 is a Na+/K+ selective light-gated ion channel, which can be inhibited by moderate concentrations of Ca2+ ions (~ 2 mM) (Zabelskii et al. 2020). Besides that, we were able to demonstrate that, upon illumination, VirChR1 is able to drive neural firing. Our efforts in the electrophysiological characterization of the VirR2 group did not result in observing any measurable photocurrents.

In order to gain more insight into the molecular function of viral rhodopsins, we expressed, purified, and characterized OLPVR1 and VirChR1 rhodopsins from the VirR1 group, and OLPVRII rhodopsin from the VirR2 group. Upon light illumination, OLPVRII rhodopsin undergoes a photocycle with 70 ms duration, which indicates pump-like behavior, whereas, both OLPVR1 and VirChR1 have channel-like photocycle with a duration of around several seconds. OLPVR1 and OLPVRII proteins were crystallized using *in meso* crystallization method and have yielded high-resolution structures of 1.4 Å and 1.9 Å respectively. Due to the conservativity of viral rhodopsins, the structures provide structural insight into their potential function. OLPVRII forms a pentamer, with a symmetrical, bottle-like central channel with a narrow vestibule in the cytoplasmic part covered by a ring of 5 arginines, whereas 5 phenylalanines form a hydrophobic barrier in its exit (Bratanov et al. 2019). The putative central channel is blocked by a hydrophobic tail of lipid from the crystallization matrix that potentially prevents the channel-like function of the protein. OLPVR1 crystallizes as a monomer and shares many structural features with well-studied channelrhodopsin 2 from *Chlamydomonas reinhardtii* (Volkov et al. 2017). OLPVR1 has three consecutive constriction sites that facilitate ion transport upon photon absorption. The OLPVR1 protomer has short extracellular loops, which sharply differentiates it from other channelrhodopsins that typically have large N- and C-terminal domains (Ernst et al.

2014). We are currently looking for ways to improve the plasma membrane localization of viral rhodopsins that can help to understand the function of OLPVRII and help viral rhodopsins to find their niche in optogenetics applications.

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SA13

The SARS-CoV-2 accessory protein Orf3a is not an ion channel, but does interact with trafficking proteins

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The severe acute respiratory syndrome associated coronavirus 2 (SARS-CoV-2) and SARS-CoV-1 accessory protein Orf3a colocalizes with markers of the plasma membrane, endocytic pathway, and Golgi apparatus. Some reports have led to annotation of both Orf3a proteins as viroporins. Here, we show that neither SARS-CoV-2 nor SARS-CoV-1 Orf3a form functional ion conducting pores and that the conductances measured are common contaminants in overexpression and with high levels of protein in reconstitution studies. Cryo-EM structures of both SARS-CoV-2 and SARS-CoV-1 Orf3a display a narrow constriction and the presence of a positively charged aqueous vestibule, which would not favor cation permeation. We observe enrichment of the late endosomal marker Rab7 upon SARS-CoV-2 Orf3a overexpression, and co-immunoprecipitation with VPS39. Interestingly, SARS-CoV-1 Orf3a does not cause the same cellular phenotype as SARS-CoV-2 Orf3a and does not interact with VPS39. To explain this difference, we find that a divergent, unstructured loop of SARS-CoV-2 Orf3a facilitates its binding with VPS39, a HOPS complex tethering protein involved in late endosome and autophagosome fusion with lysosomes. We suggest that the added loop enhances SARS-CoV-2 Orf3a's ability to co-opt host cellular trafficking mechanisms for viral exit or host immune evasion.

SA14

Autonomic control of body temperature and blood pressure in women: overlap of integrative mechanisms

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Ongoing concerns regarding climate change have increased questions regarding the potential for differences between men and women in the risk of heat illness during exertional heat stress. Interestingly, autonomic control mechanisms contributing to the regulation of body temperature and the regulation of arterial blood pressure have significant overlap in humans. This includes central autonomic control in the hypothalamus as well as peripheral control of blood flow. Mechanisms by which estradiol affects central and peripheral autonomic mechanisms result in conditions that favor both heat dissipation and lower arterial pressure. This is likely an adaptive effect in terms of maintaining low / normal resting blood pressure - that is, young women are less likely to become hypertensive compared to men. Similarly, conditions of high estradiol are often associated with increased heat dissipation (sweating / skin blood flow) and lower body temperature. However, conditions favoring lower blood pressure and increased skin blood flow can decrease orthostatic tolerance – which can also contribute to collapse in the heat. Menopause is associated with higher resting blood pressure and increased risk of hypertension. Older people also have increased risk of heat illness due to changes in thermoregulatory mechanisms, which, in women, are in part due to loss of circulating reproductive hormones. Some of the overlapping mechanisms associating estradiol with lower blood pressure and lower body temperature include beta-adrenergic receptors on peripheral blood vessels and increased nitric oxide-mediated vasodilation. Practical implications for women in a range of occupational settings are currently being investigated, including influences of common types of contraception which provide varying concentrations of exogenously administered estrogens and/or progestins.

SA15

Physiological adaptations to heat stress in women: potential “advantages” and “disadvantages” relative to men

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Sex differences in physiological responses to heat stress have been a “hot topic” over recent years, in particular with regard to risk of developing exertional heat illnesses and the important countermeasure of heat acclimation. Heat acclimation is the process through which the body garners adaptations from systematic, repeated heat exposure. These adaptations primarily include lower core body temperature (Tcore), lower heart rate (at rest and during exercise), increased sweating rate, and increased plasma volume. Mechanistically, we know there are differences between men and women in thermoregulatory responses to exercise-heat stress. Some of these differences result from physical and anthropometric differences between the sexes. For example, men are often larger and thus have lower body surface area (BSA) to mass ratio (BSA/mass^{-1}). This is an important biophysical factor because in individuals with lower BSA:mass^{-1}, heat dissipation (i.e. via sweating from the skin surface) may be limited relative to heat production (i.e. heat produced from skeletal muscle mass contraction during exercise). This physical difference may benefit women in certain environments. Additionally, female sex hormones influence thermoregulation with progesterone increasing the thermoregulatory setpoint by ~0.3-0.5°C, and estradiol increasing nitric oxide mediated vasodilation. This increase in Tcore by progesterone, during acute heat stress, does not appear to be an obstacle for women, and may prove beneficial in the acclimation process (more research is currently needed to elucidate the possible impact). The estradiol-mediated increase in vasodilation is beneficial in terms of thermoregulation, allowing for increased heat dissipation during exercise and/or heat stress. Due to the increasing utilization of hormonal contraceptives, both short and long-acting, that exogenously supplement estrogens and progestins, more work is needed to evaluate the impact of such exogenous hormonal administration on thermoregulatory processes and adaptations, including heat acclimation.

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SA16

Using Exercise Physiology to address gender health inequalities in climate change and occupational health research.

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The health impacts of climate change are already being felt by vulnerable communities, particularly in the Global South. It is widely purported that women in the Global South will be more adversely affected by climate change than men, yet there remains a dearth of empirical evidence to confirm or challenge this idea. Somewhat similarly, in human performance and occupational health literature, women were assumed to be more susceptible to the ill effects of excessive heat exposure (i.e., exertional heat stroke) based on limited empirical evidence that is now being challenged. Using a multi-year cohort study on industrial agricultural workers in Central America (the Adelante Initiative) as a case study, this session will discuss how sex-related physiological differences and lessons learnt from exercise physiology research can inform occupational health outcomes in male and female working populations in the Global South.

Case study: The Adelante Initiative began in 2017 in response to the Central American epidemic of chronic kidney disease of non-traditional origin, which is highly prevalent in agricultural workers in this region. The primary aim of this Initiative was to assess how a rest, shade, hydration intervention programme impacted the heath and work conditions of sugarcane workers at one of the largest sugarcane mills in Central America (Ingenio San Antonio). Heat stress, kidney health outcomes and workload in workers performing manual outdoor jobs (e.g., burned cane cutting, seed cutting, drip irrigation repair) were assessed longitudinally (2017-present). Very few females are currently employed as burned cane cutters at ISA. However, females are increasingly being employed in other strenuous outdoor work (i.e., seed cutting) and consequently are exposed to occupational heat stress and its associated health risks. Initial data indicates that females work at a higher physiological workload than their male counterparts. Due to a limited sample size, it is unclear if females in this work context suffer a higher incidence of kidney injury or other heat-related illness than men.

The introduction of women into a susceptible workforce such as industrial sugarcane workers, provides a unique opportunity to assess biological sex-differences in heat-related illnesses/injuries and thus gain further insight into the aetiology of diseases such as chronic kidney disease of non-traditional origin. In workforces exposed to occupational heat stress, population-level physical differences and biological differences between men and women should be factored into exposure assessments and workplace interventions. Male:female workforce ratios, particularly in jobs historically dominated by one gender, provides further information on who is at risk, what personal factors are most relevant and therefore, what interventions are the most practically beneficial. To address gender health inequalities in climate change and occupational health research it is imperative that we make every effort to include women in ongoing and future research.

SA17

Female thermal sensitivity across the life span: a hot journey

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Global warming is now the greatest threat to human prosperity and survival. Hot weather and heat extremes severely limit people's work and exercise capacity, with consequent detrimental effects on individuals' health, comfort, and productivity [1]. Undoubtedly, adjusting our thermoregulatory behaviour represents the most effective mechanism to maintain thermal homeostasis and ensure heat stress resilience [2]. Remarkably, our thermal behaviour is entirely dependent on the ability to detect variations in our internal (i.e., body) and external environment, via sensing changes in skin temperature and wetness.

In the past 30 years, we have seen a significant expansion of our understanding of the molecular, neuroanatomical, and neurophysiological mechanisms that allow humans to sense temperature and wetness [3]. However, we still lack a comprehensive understanding of how autonomic, perceptual, and behavioural responses to heat vary at an individual level, for example as a function of sex, age, and hormonal status.

Women are a group of individuals that undergo unique morphological, physiological, and hormonal changes across the lifespan. For example, consider the impact of the menstrual cycle, pregnancy, and menopause, all of which are accompanied by both short- and long-term effects on female body temperature regulation, heat tolerance, thermal sensitivity, and comfort. Surprisingly, women have been largely unrepresented in heat stress research. Indeed, a recent review highlighted that only 12-18% of participants in thermoregulation research were female over the last decade [4].

Empirical evidence indicates that innate differences in skin thermal and wetness sensitivity may exist between men and women, and this could underlie divergent behavioural responses to heat stress between these groups [5]. However, knowledge on how thermal and wetness sensitivity may vary across women's life cycle, and the implications that this may have for female thermal behaviours under heat stress, continue to be lacking. This knowledge gap provides a significant barrier to develop interventions (e.g. personalised cooling) and solutions (e.g. body-mapped sport garments) that meet the thermal needs of females across different life stages and facilitate the maintenance of an active lifestyle.

This symposium talk will review both established and novel evidence on the peripheral and central neurophysiological mechanisms underpinning skin thermal and wetness sensitivity in women, as well as their role in driving female thermal behaviours. It is hoped that this overview will stimulate the development of testable hypotheses to increase our understanding of the behavioural thermal physiology of women across the life span and at a time of climate change.

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SA18

Studies of the distribution of CFTR-rich Ionocytes in mouse airway epithelium

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Ionocytes are a new type of rare airway epithelial cells that express approximately 50% of *Cftr*-transcripts in the mouse airways. They are characterized by the expression of *Ascl3* and *Foxi1* transcription factors, which have been used to identify this cell type in the airway epithelium and submucosal glands of both humans and mice. However, their function and precise localization remain largely unknown. In this study, we aimed to investigate the distribution of ionocytes in the mouse airway epithelium.

Mice were bred in the C57Bl6/J background and maintained in the Specific Pathogen Free mouse facility of Centro de Estudios Científicos (CECs) with access to food and water ad libitum. We used 6 and 8-week-old wild-type and *Cftr*$^{\Delta F508/\Delta F508}$ animals. The trachea was splitted in two sections: the upper trachea containing the submucosal glands and the rest of the lower trachea, down the cricoid cartilage, sliced in 5µm paraffin sections in the transverse and frontal plane respectively. Ionocytes were identified by immunofluorescence against the FOXI1 transcription factor. All values were expressed as mean±S.E.M. All animal procedures were approved by the institutional IACUC (CECS-2022-03).

We found that FOXI1+cells had a triangular shape with a basolateral process. In the lower trachea the number of cells decreased towards the distal part (proximal= 2.4 ± 0.3 vs distal= 0.8 ± 0.2 FOXI1+cells mm^{-1} basal lamina, n=5, p=0.005, t-test), and were not found in the intrapulmonary airways. FOXI1+cells were more often observed in the epithelia around the collecting duct exit, in the collecting duct epithelium and in the serous acini of the submucosal glands. The total number of FOXI1+cells per millimeter of basal lamina was higher in the airway epithelium of upper trachea than in the lower trachea (6.5± 0.6 vs 4.0 ± 0.6 FOXI1+ cells mm^{-1} basal lamina, respectively; n=3, p=0.004, Rank Sum test). In general, FOXI1+cells were often present in the epithelia on top of the annular ligaments (61.7% ± 3.0; n=5; p=0.008, Rank Sum Test).

Preliminary analysis of *Cftr*$^{\Delta F508/\Delta F508}$ tissues indicated that there were no differences in the amount of FOXI1+cells when compared to wild-type lower tracheas (2.0 ± 0.7 vs 1.9 ± 0.1 FOXI1+cells mm^{-1} basal lamina, respectively, n=2 each group). Unexpectedly, we observed that FOXI1+cells were lower in issues of 6-week-old than in those obtained from 8-week-oldwild type mice (2.0 ± 0.5 (n=2) vs 4.0 ± 0.6 (n=5) FOXI1+cells mm^{-1} basal lamina, respectively, p= 0.078; Rank Sum Test).

In conclusion, our study provides new insights into the localization and distribution of ionocytes in the mouse airway epithelium. Our results indicate that ionocytes may play a role in regulating mucus composition in upper airways. We suggest that age-dependent changes in cell quantity might reflect the need of increased CFTR function in adult stages. Further research is needed to fully understand the function of ionocytes in the airway and their potential role in respiratory diseases such as cystic fibrosis.

SA19

Morphological, molecular and functional analysis of airway epithelial cell types

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The airway epithelium, the first barrier against pathogens, is endowed with active antimicrobial mechanisms and consists of different cell types, from the most abundant ciliated, goblet and basal cells to rare pulmonary neuroendocrine and tuft cells. Recently, different studies have revealed the existence of a new rare cell population, named ionocytes (1-2), characterized by a high expression of the CFTR chloride channel and the transcription factor Forkead Box I1 (FOXI1). In cystic fibrosis (CF), impaired CFTR function results in dehydration of airway surface, mucus accumulation and bacterial colonization. The airway epithelium is also the entry site for a variety of viruses, like SARS-CoV-2, who enters the cells by binding to the angiotensin-converting enzyme 2 (ACE2) (3). Interestingly, CF patients, who are particularly sensitive to respiratory viral infections, do not seem to be at risk of severe COVID-19.

Our general aim is to investigate the composition of the airway epithelium under health and disease. Specifically, we are investigating 1) expression and role of ionocytes in transepithelial ion transport; 2) ACE2 expression in CF and non-CF cells to understand whether a different expression explains the apparent resistance of CF patients to SARS-CoV-2.

Our molecular and functional studies are based on nasal and bronchial cells from CF patients and control individuals, freshly collected or cultured as differentiated epithelia.

We analyzed by immunofluorescence nasal cells from a broad panel of CF and non-CF patients. Ionocytes were easily detected in both sample types as FOXI1-positive cells and appeared more abundant in the nasal (3-5%) compared to bronchial epithelium. CFTR expression at the plasma membrane correlated with the type of CF mutation: patients with severe mutations (affecting CFTR synthesis or trafficking), showed absent or markedly decreased expression in the plasma membrane. In contrast, patients with milder mutations exhibited a clear CFTR signal in the apical membrane. In general, we found no enhanced abundance of ionocytes in CF individuals with severe CFTR mutations, which could be expected as a compensatory mechanism for the defect in CFTR function. We conducted similar analysis in patients with primary ciliary dyskinesia, another genetic disease with defective mucociliary clearance and susceptibility to bacterial infection. We detected no significant differences compared to control individuals.

Regarding ACE2, we found a substantial higher expression in nasal vs. bronchial cells. Interestingly, ACE2 appeared to be specifically localized on the apical membrane of ciliated cells, at the base of cilia. Furthermore, we found no different ACE2 expression between CF and non-CF samples, thus in contrast with the results of a recent study that reported a decreased ACE2 expression in CF epithelia (4).

The role of ionocytes in airway epithelia is still unclear. The high expression of CFTR may imply a prevalent role in chloride secretion. However, since other more abundant cell types in the

epithelium also express CFTR, it is possible that CFTR in ionocytes have a more specialized function. The higher resistance of CF patients to severe forms of COVID19 does not correlate with lower ACE2 expression. Further studies are needed to clarify the underlying mechanism.

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SA20

Pulmonary Neuroendocrine Cells: Rare, but not Dispensable

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Pulmonary neuroendocrine cells (PNECs) represent less than 1% of airway epithelium. Aside from being precursors for small lung cancer cells, whether they play a role in normal lung function remains poorly understood. Our findings show that PNECs are essential airway sensors that perceive and respond to aerosol signals. They are essential for amplifying allergen-induced asthmatic response. When increased in number, they produce excess neuropeptides which disrupts endothelial barrier, resulting in accumulation of fluid in lung and respiratory distress. These findings illustrate the multiple facets of PNEC function in homeostasis and disease.

SA21

Calcium Cycling in the Avian Heart: the missing link in vertebrate cardiac evolution

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Bird cardiomyocytes are long, thin and lack t-tubules, similar to ectothermic non-avian reptiles. Yet, birds achieve greater contractile rates and developed pressures than mammals, whose wide cardiomyocytes contain a dense transverse (t)-tubular network allowing for uniform excitation-contraction coupling and strong contractile force. To address this apparent contradiction, this talk will link recent electrophysiological studies on bird cardiomyocytes with ultrastructure measurements and computational approaches. Data will show that the strong transsarcolemmal Ca^{2+} influx via the L-type Ca^{2+} current (I_{CaL}) and the high gain of Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR), coupled with the internal SR Ca^{2+} release relay system, facilitates the strong fast contractions in the long thin bird cardiomyocytes, without the need for t-tubules. The significance of this in relation to the evolution of the vertebrate heart and the evolution of endothermy will be discussed.

Shiels HA. Avian cardiomyocyte architecture and what it reveals about the evolution of the vertebrate heart. Philosophical Transactions of the Royal Society B. 2022 Nov 21;377(1864):20210332.

SA22

Pacing intracellular Ca2+ signals in exocrine cells

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The major physiological function of exocrine acinar cells from the pancreas and salivary gland is the secretion of proteins and fluid which are initiated by changes in cytosolic Ca^{2+} following neurotransmitter or hormone exposure. It is established that the spatiotemporal characteristics of the Ca^{2+} signal are vitally important for the appropriate stimulation of secretion and these properties are often disrupted in disease states. Experiments performed in isolated tissue have documented the complexity of these signals including sub-cellularly restricted signals, intra and inter-cellular Ca^{2+} waves, and apparent pacing of signals within individual acinar clusters by initiator cells. Whether these characteristics are mirrored *in vivo* was not known. To address this question, we have generated mice expressing the genetically encoded Ca^{2+} indicator GCamp6f specifically in acinar cells and developed an imaging platform to study the characteristics of Ca^{2+} signals *in vivo* in anesthetized mice by multi-photon microscopy. In submandibular salivary acinar glands (SMG), we show that stimulation of intrinsic nerves to the gland result in rapid oscillatory Ca^{2+} signals following ACh release. These events are positively correlated with fluid secretion. These signals appear to initiate in specific cells within individual acini and propagate to neighboring cells. In pancreas, Ca^{2+} signals were observed following neural stimulation that were dependent on ACh release. Ca^{2+} signals as a function of elevated serum cholecystokinin were observed in fasted animals that were augmented in terms of the number of responding cells and peak response following feeding. Both nerve stimulation and CCK induced Ca^{2+} oscillations in pancreatic acini, but with markedly distinct temporal and spatial characteristics. We speculate that initiating cells in each gland are more directly stimulated, either by direct neural innervation or by proximity to the vasculature or alternatively represent cells most sensitive to secretagogue by virtue of receptor number. In total, these studies define the physiological characteristics of Ca^{2+} signals *in vivo* and the platform will be useful in future investigation of disruption of Ca^{2+} signaling in disease states of exocrine tissue.

SA23

Origin of rhythmicity in the bladder and urethra

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The smooth muscle organs of the lower urinary tract comprise the bladder detrusor smooth muscle (DSM) and internal urethral sphincter, which have a reciprocal contractile relationship during urine storage and micturition. As the bladder fills with urine, DSM remains relaxed to accommodate increases in intravesical pressure while urethral smooth muscle cells (USMC) generate sustained tone to occlude the urethral orifice, preventing leakage. Upon onset of micturition, this contractile behaviour reverses, as USMC relax, allowing passage of urine from the bladder, which contracts to expel urine via the now open urethra. While neither of these organs displays uniform coordinated regular contractions, similar to phasic tissues such as the small intestine, lymphatics or renal pelvis, they do exhibit certain patterns of rhythmicity at cellular and tissue levels which underly their physiological function. In rabbit and guinea-pig urethra, regular electrical slow waves are recorded from circular USMC. This activity is linked to specialized populations of pacemaker cells expressing vimentin, c-kit and Ca^{2+}-activated-Cl^- channels, like interstitial cells of Cajal (ICC) in the gastrointestinal (GI) tract. While contractions of urethral muscles do not manifest as coordinated phasic contractions, in these species ICC-like cells might pace individual USMC bundles (through activation of voltage-gated Ca^{2+} channels) to contract asynchronously, with contractions of multiple bundles summating as tone. In mice, USMC are indeed rhythmically active (firing propagating Ca^{2+} waves linked to contraction), and this rhythmicity is asynchronous across the tissue, summating to form tone. However, experiments in mice have failed to demonstrate a voltage-dependent mechanism for regulating this rhythmicity or contractions *in situ*, suggesting that urethral tone results from intrinsic abilities of USMC to 'pace' their own Ca^{2+} mobilization to generate Ca^{2+} waves required for contraction. During the filling phase, animal and human bladders exhibit small transient increases in intravesical pressure, brought about by locally propagating transient contractions of the bladder wall. These transient contractions are critical in regulating sensory afferent activity – relaying sensations of bladder fullness to the CNS. *Ex-vivo* DSM strips exhibit spontaneous rhythmic contractions, mimicking transient concentrations observed during filling *in-vivo*. While DSM spontaneous contractions appear to an intrinsic myogenic property, they are regulated by autonomic nerves and urothelium. Action potentials and associated rises in DSM cytosolic Ca^{2+} are essential for generating these contractions, with this activity appearing to be voltage dependent. The presence of putative 'pacemaker' interstitial cells in the DSM layers have been controversial. Similar, to the GI tract and urethra, Kit^+ cells are present in the DSM layer, however, unlike these other organs these Kit^+ cells are almost exclusively mast cells and thus unlikely to serve as pacemakers. However, another interstitial cell with immunopositivity for antibodies against PDGFRα, has recently been suggested to regulate DSM excitability by potentially serving as mechano and neural transducers, through activation of inhibitory purinergic-SK3 pathways. While the mechanics of rhythmic or tonic contractions in both bladder and urethra is myogenic, there are clear disparities in the cell types, molecular pathways and mechanisms of coordination that lead to these physiological behaviours in both organs.

SA24

Peristaltic pacemakers of the upper urinary tract

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Urine expulsion from the upper urinary tract is a necessary process that eliminates waste, promotes renal filtration, and prevents nephron damage. To facilitate the movement of urine boluses throughout the upper urinary tract, smooth muscle cells that line the renal pelvis contract in a coordinated effort to form peristaltic waves. Resident pacemaker cells in the renal pelvis are critical to this process and spontaneously evoke transient depolarizations that initiate each peristaltic wave and establish rhythmic contractions. This talk will discuss the mechanisms responsible for pacemaking and our current methods to improve the identification of pacemaker cells. Until recently, renal pacemakers have been termed "atypical smooth muscle cells" due to their low expression of smooth muscle myosin and poor organization of myofilaments compared to "typical smooth muscle cells" that perform peristalsis. Our group discovered that pacemaker cells also express the tyrosine kinase receptor PDGFRα, enabling their identification and purification amongst other renal pelvis cell types. Employing our improved identification methods, we have determined that the calcium-activated chloride channel, ANO1, is expressed by pacemaker cells and may contribute to spontaneous depolarization. A greater understanding of pacemaker and peristaltic mechanisms is warranted since aberrant contractile function may underlie diseases such as hydronephrosis, a deleterious condition that can cause significant and irreversible nephron damage.

SA25

The use of ex vivo human serum to study age and chronic inflammatory disease related muscle cell atrophy

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Cell-based models of ageing and disease provide an important platform to probe and extend the mechanistic insights of muscle cell atrophy from invasive and logistically challenging human trials. While cell-based models provide useful insights into the role of metabolic pathways, translation from cell to human can be limited by non-physiological culture conditions, supraphysiological treatment dosages and experimental conditions targeting a single protein, or receptor. Over recent years, there has been an increase in research aimed at improving the physiological relevance of in vitro work, including the use of human plasma and serum. The ex vivo co-culture model has the potential to create a systemic environment representative of ageing and chronic inflammatory disease states. Recent advancements in the application of the ex-vivo co-culture model have highlighted the capacity of human serum to induce atrophy in relation to its host environment. We, and others have utilised the human serum and plasma from young and old males, to investigate ageing-related cellular atrophy (Kalampouka *et al.*, 2018; Allen *et al.*, 2021) and chronic liver disease patients to investigate disease related atrophy (Allen *et al.*, 2022). This talk will describe the effects of young and old ex vivo serum on cellular growth and protein stasis, before outlining the utility of the ex-vivo model to investigate muscle atrophy in chronic inflammatory disease conditions e.g., chronic liver disease and rheumatoid arthritis.

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SA26

The effect of female sex hormones on human skeletal muscle metabolism – an ex vivo/in vitro approach

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In vitro models have long been used to further our understanding of skeletal muscle metabolism. Using these methods, researchers can study the mechanistic response to various stimuli (e.g., nutrient exposure/withdrawal, electric stimulation etc) that may be difficult to achieve or isolate in large human trials. Supraphysiological treatment conditions are often used on immortalised cells (often not derived from humans) and create an even more artificial milieu – rendering it difficult to translate *in vitro* findings to humans.

In recent years, there has been an ever-growing focus on the potential presence of sexual dimorphism in various aspects of muscle physiology, however, to date there has been little specific focus on female sex hormones. Female physiology is characterized by fluctuations in hormone levels throughout the menstrual cycle, such that oestrogen levels peak during the late follicular phase while progesterone levels are highest during the mid-luteal phase. Skeletal muscle expresses both oestrogen and progesterone receptors (1), and oestrogen has been purported to 'protect' muscle from exercise induced muscle damage via several different mechanisms in animal models (2). Moreover, progesterone treatment impairs insulin stimulated glucose metabolism in rodents implying a direct effect of progesterone on skeletal muscle that warrants further investigation (3).

In this talk, I will describe the findings from recent experiments whereby immortalised human skeletal muscle cells (4) were utilised to understand the effects of female sex hormones on skeletal muscle anabolism. To further enhance the translatability of such findings, serum samples obtained at different phases of the menstrual cycle (early follicular phase - low oestrogen/progesterone, late follicular phase - high oestrogen and mid luteal phase - high oestrogen/progesterone) were applied to cells to allow physiologically relevant concentrations/ratios of these hormones to be studied. Such a model can therefore provide a greater depth of understanding of the role of female sex hormones on human skeletal muscle anabolism.

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SA27

A translational model of muscle protein synthetic bioactivity using ex vivo human serum

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Background:

In vitro models provide an important platform for the investigation of skeletal muscle growth to inform and extend mechanistic insights in invasive and often logistically challenging human trials. Although these models allow for greater understanding of the mechanistic underpinning of adaptation in skeletal muscle, many models involve supraphysiological dosages and non-physiological conditions which limit translation of findings to humans. The aim of this research was the development and validation of a translational model for the evaluation of sustainable protein sources in stimulating muscle protein synthesis (MPS) and skeletal muscle anabolism using *ex vivo* human serum. To achieve this overall aim, three primary objectives had to be realised: (i) Development of an *in vitro* skeletal muscle cell bioassay to measure muscle growth and MPS; (ii) Development of an *ex vivo* model to evaluate the humoral effect on MPS in response to protein feeding; (iii) Use of a stable isotope technique to evaluate MPS in response to protein feeding *in vivo.*

Methods:

Changes in cell behavior and adhesion properties were monitored by measuring impedance via interdigitated microelectrodes using the xCELLigence system. MPS was measured by puromycin incorporation using the SUnSET technique, intracellular signalling measured by western blot, and myotube thickness by microscopy. To establish the ability of the bioassay to measure the humoral effect of MPS in response to protein feeding, media was conditioned by *ex vivo* human serum from fasted, and protein-fed conditions. To evaluate MPS in response to protein feeding *in vivo,* acute MPS (5 h) was assessed by measuring stable isotope deuterium oxide (D_2O) incorporation into m. vastus lateralis skeletal muscle following consumption of sustainable proteins compared with a non-essential amino acid (NEAA) formulation.

Results:

In this presentation we will demonstrate the ability to monitor changes in cell behaviour, cell size and intracellular signalling when conditioning media with *ex vivo* human serum in response to feeding with alternative proteins. Proteins containing essential amino acids, known regulators of MPS and muscle anabolism, display greater anabolic qualities than isonitrogenous NEAA formulations. We also confirm translation of this in a human *in vivo* model using stable isotope tracers.

Conclusion:

We have developed a translational model of muscle protein synthetic bioactivity using *ex vivo* and *in vivo* methodologies. We have shown that we can impact MPS *in vitro* using *ex vivo* human serum to condition media, that MPS *in vitro* is differentially regulated by *ex vivo* serum

from alternative proteins compared with an isonitrogenous NEAA control, as well as translation of these findings *in vivo* using stable isotope technology.

SA28

SPOCs, video feedback, and finding benefits from ed tech where others do not look

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Distant and online education has been around since long before the pandemic forced every teacher to consider this form of delivering education. Therefore, we can draw upon existing evidence to determine what works and what doesn't, from long before 2020. In the hectic of the pandemic, less informed choices led to very different experiences with online education of both teachers and students. During this presentation we will move from MOOCs to SPOCs (Small Private Online Courses). We will delve into scalability and focus on deep learning, and social presence as ways to make online education a rich experience. Forms of providing feedback in an online environment will be discussed, including different actors in feedback, and the learnings from both receiving and providing feedback. From feedback, we will naturally move towards assessment. We will discuss assessment *for* learning, assessment *of* learning and assessment *as* learning in the context of online education. Also here, students' new best friend ChatGPT will be included in our discussion on online education and online assessment.

Technologies in Biomedical and Life Sciences Education
(https://link.springer.com/book/10.1007/978-3-030-95633-2)

SA29

Pandemic positives - how technology changed how we teach and assess, and what does the next challenge look like.

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In March 2020 the COVID-19 pandemic led to a huge shift in teaching and assessment across the HE sector. Instead of in class sessions,institutions around the world moved teaching online. For lectures, this typically meant delivering content via asynchronous resources such as videos, supplemented by synchronous, online active learning sessions, which allowed students to consolidate knowledge and develop problem solving skills. Assessments moved away from invigilated examinations, to online, open book exams, and this meant a change in the types of assessments being used as well (away from recall and towards application). Many of the changes implemented during this time had positive benefits for students, and as the sector transitioned back to in class, these inclusive approaches were retained. Practitioners retained the use of flipped learning, supported by active sessions. Many assessments did not go back to invigilated exams, but stayed online and open book. All of these approaches provide a more inclusive approach, recognising that our student cohorts are diverse. However, the sector is about to undergo yet another shift, with the increased prominence of AI technology. While not the size of shift we saw with COVID-19, it is clear that if we are to retain the inclusivity benefits of having a range of assessments, then we are going to have to look carefully at how we design those assessments. We are also going to have to evaluate how we prepare our students for future career pathways where the targeted use of AI is becoming more frequent.

SA30

Non-invasive evaluation of skeletal muscle oxidative function in vivo in health and disease: an exercise physiology perspective by near-infrared spectroscopy.

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In most activities related to work or leisure the energy for muscle work substantially comes from oxidative metabolism. Impairments of this metabolism can significantly affect exercise tolerance and performance, may significantly affect the patient's clinical picture and quality of life, and represent an important predictor of mortality. Near-infreared spectroscopy (NIRS) can offer insights into the physiological and pathophysiological adaptations to conditions of increased O_2 needs which involve, in an integrated manner, different organs and systems of the body. In terms of patient evaluation, NIRS allows to determine the evolution of the functional impairments, identifies their correlations with clinical symptoms, evaluates the effects of therapeutic or rehabilitative interventions, and allows to to gain pathophysiological insights[1,2].

Strengths and limitations of NIRS have been discussed in recent reviews[1,3]. Skeletal muscle fractional O_2 extraction (SMFOE), the main variable evaluated by NIRS, is conceptually homologous to arterial-venous O_2 concentration difference, and is the result of the dynamic balance between O_2 utilization and O_2 delivery in the tissue under consideration[1,3]. The reduced peak SMFOE during an incremental exercise identified and quantified the incapacity to increase O_2 extraction (one of the key pathophysiological mechanisms of these diseases) in patients with mitochondrial myopathyes or McArdle disease[2]. SMFOE allowed, in these patients, insights into the mechanisms responsible for the positive effects of exercise training,[2] and in McArdle patients into the pathophysiology of the “second wind”[2]. Impairments of oxidative metabolism, expressed as reduced SMFOE peak, were described in several other pathological conditions[1] and after exposure to bed-rest/microgravity and/or hypoxia[4].

The slope of the linear SMFOE increase at intermediate work rates, during an incremental exercise, allowed inferences in the adequacy of O_2 delivery in patients with chronic heart failure[5] or in heart transplant recipents[6].The plateau of SMFOE at high work rates has been the object of active research in terms of its associations with variables such as critical power, maximal lactate steady state, respiratory compensation point[7].

SMFOE during the rest-to exercise transition was utilized to evaluate the adequacy of the adjustment of microvascular O_2 delivery *vs.* that of O_2 uptake, which was impaired in patients with chronic heart failure[1], metabolic myopathies[2], in subjects exposed to nicrogravity/bed-rest[8].

During a transient muscle ischemia obtained by cuff inflation, the rate of deoxygenation determined by NIRS indicates muscle $V'O_2$[3]. By adopting rapid inflation-deflation protocols during the recovery from exercise, NIRS allowed to determine muscle $V'O_2$ off-kinetics, mirror image of [PCr] kinetics and a classic index of functional evaluation of oxidative metabolism[9]; studies have been performed in patients[9], healthy subjects[9-10], subjects exposed to microgravity/bed-rest[11]. A modification of the rapid inflation-deflation protocol allowed to specifically investigate peripheral O_2 diffusion[12]. The rate of reoxygenation following a transient muscle ischemia evaluates the microvascular response to an ischemic stress (“reactive hyperemia”)[3]. Insights into peripheral O_2 diffusion can be obtained by analysis of changes of the

total (oxygenated + deoxygenayed) Hb signal, reflecting changes in capillary hematocrit[3]. Exciting new perspectives (simultaneous measurements of microvascular blood flow, SMFOE and regional oxidative metabolic rate) have been raised by diffuse correlation spectroscopy[13].

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SA31

Measuring cerebrovascular function in humans in response to dietary interventions

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Flavonoids are small molecules that can be found ubiquitously in plants (e.g. cocoa, berries, grapes, apples) and can protect humans against vascular disease, as evidenced by improvements in peripheral endothelial function, likely through nitric oxide (NO) signalling. Emerging evidence also suggests that diets rich in these compounds may protect against cognitive decline later in life, but the underlying mechanisms are not well established.

We have conducted randomized, counterbalanced, double-blind, placebo controlled, within-subject acute studies in healthy young adults to investigate the effects of one single dose of cocoa flavonoids (flavanols) on prefrontal cortical oxygenation using Near Infrared Spectroscopy (NIRS). In our first study (N=18), we showed that flavanol intake leads to faster (approx. 1 min; $p < 0.001$) and greater brain oxygenation ($p=0.030$ *for Oxygenated Haemoglobin,* O_2Hb) in response to hypercapnia (5% CO_2), as well as higher performance in a Stroop Task, only when cognitive demand is high ($p=0.045$). We further observed that only participants who benefitted from flavanol intake during hypercapnia, also demonstrated improvements during cognitive performance (1). More recently we have also shown that cocoa flavanols might be beneficial in the context acute mental stress. During periods of stress, individuals often increase their consumption of unhealthy foods, especially high fat foods, and it is well established that both fat and mental stress alone can negatively impact peripheral vascular function. However, their effects on the cerebral vasculature are less understood. We have firstly demonstrated that a high-fat breakfast (56.5 g fat) impaired prefrontal cortical oxygenation ($p<0.05$ *for* O_2Hb *and Tissue Oxygenated Index, TOI*) during the mental stress episode in healthy young volunteers, in comparison to a low fat-breakfast (11.4 g fat) (N=19). In a follow-up study (N=23), we have further investigated whether a high-fat breakfast administered with an acute dose of cocoa flavanols may prevent fat-induced impairments in cortical oxygenation during stress. We are currently analysing this set of data and will be able to share at the meeting.

Together our data suggests that flavonoid-rich foods might be an effective dietary strategy to improve cortical oxygenation in young healthy adults and that might be important in the context of high cognitive demand and during periods of stress. These findings will have important implications for future research to explore the relationship between food choices and cerebral haemodynamics during cognitive performance/mental stress. Our data further suggests that flavonoids might exert similar actions on the cerebral vasculature as they do in the peripheral vasculature.

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SA32

Advancing clinical physiological monitoring with state-of-the-art diffuse optics techniques

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Diffuse optics (DO) has been widely used for physiological monitoring for over 40 years [1]. Near-infrared spectroscopy (NIRS) is a well-known DO technique that measures changes in light attenuation due to changes in tissue chromophore concentrations. While NIRS instruments based on continuous waves (CW) have primarily been used to monitor muscle and brain oxygenation changes, recent methodological advancements have increased the accuracy and robustness of NIRS measurements and enabled the monitoring of metabolism and the development of new biomarkers. These advances have pushed the use of NIRS, and more generally DO, in the clinic.

During my presentation, I will showcase the work I have been conducting at the University College London (UCL) to advance the capabilities of DO and extend its use in various fields, such as neuroscience, physiological research, and particularly in a clinical environment.

Indeed, our group, led by Prof. Tachtsidis, is focused on developing optical methodologies to measure brain oxygenation and metabolism. To do so, our primary focus has been on the development of broadband NIRS infrared spectroscopy, an extension of the standard NIRS technique that uses hundreds of wavelengths to acquire more information about the brain. This technique allows us to monitor, on top of the traditional oxygenation parameters monitored with NIRS, the concentration changes in cytochrome-c-oxidase (oxCCO), which is a marker of metabolism. We have shown that monitoring both oxygenation and metabolic changes concurrently could have significant impact for clinical monitoring [2].

Secondly, I will show more recent work that has focused on combining diffuse correlation spectroscopy (DCS) with our broadband NIRS device to further extend the capacities of the system, in an instrument called FLORENCE [3]. Indeed, DCS is an established optical modality that enables non-invasive measurements of blood flow in deep tissue by quantifying the temporal light intensity fluctuations generated by dynamic scattering of moving red blood cells. Thus, the addition of DCS gives us access to blood flow information, enabling us to extract information about the cerebral metabolic rate of oxygen ($CMRO_2$). I will show the benefits of accessing all of this information in a clinical context.

Finally, I will briefly talk about the most advanced instrument that we have been developing, called MAESTROS, which is based on time-domain NIRS (TD-NIRS) and measures the arrival time of photons [4]. This is the most advanced form of NIRS and can unlock new possibilities. It is notably the best technique to enhance the depth sensitivity of NIRS measurements, enabling us to overcome the most significant issue with NIRS, i.e., superficial tissue contamination [5]. I will show an example of a clinical use of this system, together with the new possibilities that it offers.

In conclusion, my talk will provide an overview of the work done at UCL to advance the state-of-the-art of DO and promote its usefulness and adoption in a clinical context.

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SA33

Dual slopes in diffuse optics: Applications to the brain and skeletal muscle

Sergio Fantini, Angelo Sassaroli, Giles Blaney, Cristianne Fernandez, Jodee Frias, Fatemeh Tavakoli

undefined

Introduction: Near-infrared spectroscopy (NIRS) and diffuse optical imaging (DOI), in the wavelength range 600-1000 nm, have been used for non-invasive optical studies of biological tissues for a long time. Some notable applications include tissue oximetry, pulse oximetry, assessment of blood flow and oxygen consumption in skeletal muscles, functional brain imaging, and optical mammography. Besides continuous-wave (CW) methods that use constant illumination, time-resolved methods either in the time domain (TD: pulsed illumination and time-resolved detection) or in the frequency domain (FD: intensity-modulated illumination and phase-resolved detection) have been introduced. Furthermore, slope methods based on the collection of data at multiple source-detector distances have been proposed, especially with CW and FD methods, to perform absolute measurements of tissue optical properties or to minimize sensitivity to superficial tissue layers. Slope techniques are typically based on either a single source (and multiple detectors) or a single detector (and multiple sources), in which case they may be termed “single-slope” methods. A “dual-slope” approach, identified as “self-calibrating,” was introduced to perform slope measurements that are insensitive to instrumental and optical coupling effects, resulting in calibration-free measurements.

The motivation of this work is to achieve quantitative optical measurements and preferential sensitivity to deep tissue using frequency-domain NIRS (FD-NIRS) in dual-slope configurations. This is important for non-invasive optical measurements to achieve preferential sensitivity to brain and muscle tissue underneath scalp/skull and skin/adipose layers, respectively.

Methods: Theoretical calculations based on diffusion theory were first run to characterize the spatial region of sensitivity of the dual slope technique implemented with FD-NIRS and two illumination points and two collection points that realize source-detector distances of 2.5 and 3.5 cm. Homogeneous and heterogeneous media were considered, with special emphasis on two-layered media. *In vivo* results were then obtained on human subjects from the primary visual cortex during visual stimulation, and from the forearm muscle during either venous occlusion or arterial occlusion in the upper arm.

Results: In both theoretical simulations and *in vivo* measurements with FD-NIRS, we consistently found enhanced depth sensitivity using phase vs. intensity data, and using dual-slope vs. single-distance data. We also found that the relative scattering properties of superficial and deeper tissue affect the depth sensitivity achieved by different optical measurements. In the case of brain measurements, we observed the lowest sensitivity to cortical hemodynamics using single-distance intensity, intermediate sensitivity using single-distance phase or dual-slope intensity, and maximal sensitivity using dual-slope phase. In the case of muscle measurements, the different hemodynamics and oxygen metabolic rates in superficial adipose tissue and deeper muscle tissue result in quantitatively and qualitatively different dynamics observed with different data types.

Conclusions: Dual-slope measurements feature desirable aspects of practical and conceptual significance that can help advance a number of spectroscopy and imaging applications in the field of non-invasive diffuse optics. Specifically, they can provide more specific measurements of cerebral hemodynamics in functional brain imaging, and more detailed characterization of skeletal muscle hemodynamics and oxygenation during vascular occlusion and exercise protocols.

SA34

The relationship between respiratory mechanics and neural control of respiratory muscles

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The diaphragm is the major inspiratory muscle, but the intercostal and scalene muscles also generate inspiratory pressure to draw air into the lungs. There is synergistic activation of the inspiratory muscles to ventilate the lungs, but coordinated activity across these muscles is also important to reduce the work of breathing. In recent history, the mechanics of the inspiratory muscles for breathing is quantified as a fractional change in muscle length per passive increase in lung volume, i.e. mechanical advantage (De Troyer *et al.*, 2005). The relative contribution of the inspiratory muscles, and even different regions within each muscle, to generate inspiratory pressure differs. How does the central nervous system deal with the diversity and redundancy in respiratory muscle mechanics?

In humans, single motor unit studies have demonstrated that the parasternal intercostal muscles are activated in a precise pattern, with earlier and greater activity in the rostral interspaces compared to the caudal spaces during eupnoea. This finding is robust, having been replicated twice (Hudson *et al.*, 2019). This rostrocaudal pattern of neural drive mirrors the relative inspiratory mechanical advantages of these muscles. Similarly, a rostrocaudal gradient of neural drive parallels that of mechanical advantage in the external intercostal muscles. In addition, in the external intercostals, there are *within* interspace differences in neural drive and mechanical advantage, both being greatest in the dorsal portion of muscle and declining along the interspace. The coefficient of correlation between neural drive and mechanical advantage is 0.99 for both intercostal muscle groups (Hudson *et al.*, 2019). This remarkable relationship between neural drive and mechanics is a strategy that minimises the metabolic cost of muscle activation (De Troyer *et al.*, 2005) and led to the discovery of the “principle of motor unit recruitment by neuromechanical matching” (Hudson *et al.*, 2019).

For the diaphragm, neural drive is greater to the costal than the crural portion, with increases in either voluntary or involuntary drive to breathe (Nguyen *et al.*, 2020). Given the costal portion generates more thoracic expansion, via the zone of apposition (Domnik *et al.*, 2020), this suggests motor unit recruitment according to neuromechanical matching occurs across portions of the major inspiratory muscle in humans.

The ‘respiratory muscles’ have other motor functions, and their neural control adapts according to their mechanics in the motor task. In targeted voluntary breaths, where the mechanics of the intercostal muscles are comparable to eupnoea, the rostrocaudal pattern of neural drive is maintained (see Hudson *et al.*, 2019). However, in ipsilateral trunk rotation, for populations of the same intercostal motor units, the pattern of recruitment across interspaces is reversed compared to the rostrocaudal gradient during eupnoea. This is likely to reflect different mechanics for the parasternal intercostal muscles in these tasks (Hudson *et al.*, 2017).

Motor unit recruitment by neuromechanical matching is the most efficient way to recruit the respiratory muscles for breathing and other tasks in health. Non-invasive methods to assess

patterns of inspiratory muscle activity will facilitate discoveries on neuromechanical matching in clinical populations and is the focus of new research.

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SA35

Respiratory mechanics, breathlessness and exercise limitation in health and disease

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Breathlessness has been defined as “a subjective experience of breathing discomfort that consistent of qualitatively distinct sensations that vary in intensity”. It is a complex phenomenon, which is influenced by both neurophysiological and psychological factors. Breathlessness impacts upon individuals’ capacity to undertake physical activity. This can limit exercise capacity and, in severe cases, restrict the ability to mobilise, undertake daily activities and independently self-care, thereby deleteriously impacting upon health-related quality of life.

The sensation of breathlessness arises as a consequence of respiratory muscle motor activity through proprioceptive pathways. Inspiratory muscle activity increases in health during exercise, and in disease states where there is imbalance in the loads and capacity of the respiratory muscle pump. This imbalance leads to increased neural respiratory drive in the medulla. Conscious awareness of this ventilatory drive is perceived as breathlessness. It has been proposed that breathlessness intensity increases when there is mismatch between sensory afferents and efferent neural respiratory drive.

Disease states leading to load-capacity-drive imbalance can broadly be considered under the classifications of obstructive airways disease, neuromuscular and chest wall disease and obesity. In obstructive lung disease, most commonly chronic obstructive lung disease (COPD), airway inflammation, bronchospasm and sputum impose resistive loads, loss of alveolar fibroelasticity leads to elastic loading, and expiratory flow limitation with consequent intrinsic positive end expiratory pressure ($PEEP_i$) imposes a threshold load. Capacity of the respiratory muscle pump is reduced in COPD due to hyperinflation, which impairs force generating capacity. In neuromuscular and chest wall disease, respiratory muscle weakness reduces pump capacity, and upper airway obstruction, secretions and stiff lungs. In obese subjects, upper airways obstructive imposes resistive loading, reduced lung compliance contributes to elastic loading and threshold loading arises through early airway closure leading to $PEEP_i$. Capacity may be impaired through reduced functional residual capacity and ventilation:perfusion mismatch.

It is not possible to directly quantify central ventilatory drive, therefore surrogate indices are utilised. Inspiratory muscle activity increases in response to increased neural respiratory drive, and thus represents a measurable and potentially clinically valuable objective physiological marker of neural respiratory drive. Electromyography (EMG) has been implemented using invasive and non-invasive techniques amongst healthy subjects and in patients with load-capacity imbalance. This talk will provide an overview of these approaches and clinical applications of respiratory muscle EMG.

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SA36

The effects of ageing on the respiratory physiology of exercise

William Sheel

undefined

Normative aging of the respiratory system involves significant structural changes leading to a progressive decline in pulmonary function. Age-related structural changes include decreases in lung elastic recoil, airway size, respiratory muscle strength and chest wall compliance. Declines in pulmonary function are especially critical when considering the significant demands placed on the lung, airways, and respiratory musculature during conditions of dynamic exercise. Moreover, biological sex has historically not been considered within those studies designed to examine the interaction between age-related changes to the structure and function of the respiratory system and the effect on the integrated response to exercise. This presentation will first summarize changes to the respiratory system that are associated with healthy ageing. With this framework in mind, two inter-related questions will be addressed. First, what is the metabolic cost of exercise hyperpnea and how does this differ on the basis of age and sex? We have recently quantified the metabolic cost of breathing of exercise ventilations through voluntary hyperpnoea in healthy younger (23±3 y) and older (63±6 y) males and females. We found that both younger and older females have a higher cost to breathe than their male counterparts during moderate and high-intensity exercise. In addition, older individuals incur a higher cost to breathe than younger individuals for a given absolute ventilation. Second, is the pressor response during high levels of inspiratory work heightened in older adults and is there an effect of circulating ovarian hormones. Healthy, normotensive young (26±3 y) and older (64±5 y) males and females performed inspiratory pressure threshold loading to task failure. Consistent with previous reports, younger females had a lower blood pressure response to high respiratory work relative to young males. Older adults had a greater mean arterial pressure compared to young however, the sex difference was absent in older individuals. Our observations in young adults are analogous to previous work where metaboreflex responses differed by sex when limb work is performed. Likewise, we interpret the similar blood pressure response between older males and females to be related to the reduced concentration of sex hormones. Our findings point to independent effects of ageing and sex on the respiratory muscle metaboreflex responses. Collectively, our recent work speaks to the need to further understand the demands placed on the respiratory system during exercise of healthy older adults and how this may differ on the basis of sex. Understanding what constitutes ‘normal’ is especially important from a clinical perspective given that many diseases of the heart and lungs occur in older individuals.

SA37

The mechanisms of emmetropization

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One of the basic questions of myopia development is why it does not inhibit itself but rather tends to progress. This is in apparent contradiction to results of animal models, where myopia can be simulated by positive lenses and, in fact, inhibits eye growth, leading to shorter eyes and hyperopia. Together with Barbara Swiatczak at IOB, we found that also the human retina can detect myopic defocus and trigger shorter eyes, but this was possible only in emmetropic eyes while myopic eyes had apparently lost this ability. A big question is then how the emmetropic retina can detect positive defocus (note that we don't need such a function for vision). A great signal would emerge from longitudinal chromatic aberration which makes image focus more myopic in the blue, compared to the red. Comparison of focus in both planes could provide the sign of defocus. We have developed software to present movies with simulated chromatic defocus. We found that the emmetropic retina responded exactly as expected. When blue was calculated unsharp, eyes became shorter but when red was calculated unsharp, eyes became longer. Again, myopic eyes had lost this ability and only became longer. There appears to be a fundamental functional change in the myopic retina - we need to find out what it is and when it happens. An emerging question is then why various novel spectacle designs can slow myopia progression - as we found that the myopic retina does not respond to positive defocus, these glasses must stimulate the retina in a different way, other than imposing positive defocus.

SA38

Aspects of Retinal Signalling in the Myopic Eye

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Introduction: It is well accepted that reduced visual function occurs in physiological (non-pathological) myopia. However, it is less well understood whether this is due to true dysfunction in the retinal cells, or simply a result of reduced density of functionally normal cells secondary to myopic eye growth and retinal stretch. To investigate this, we considered spatial and temporal summation characteristics in physiological myopia, and compared this to our previous work in glaucoma, a pathological eye condition that causes reduced cell density (cell death) *and* cell dysfunction. Spatial and temporal summation are core visual functions which refer to how the perceptive fields of the visual system summate light over space and time respectively.

Aim: To investigate spatial and temporal summation in physiological myopia

Method: Spatial summation was investigated in 24 myopes (mean: -4.14DS, range: -0.50DS, -9.75DS) and 20 non-myopic controls (mean: +0.71DS, range: +1.75DS, -0.25DS) by measuring achromatic contrast thresholds for six stimuli varying in area (0.01–2.07 deg^2, 200ms). The effects of refractive error induced variations in retinal image size (RIS) were considered by correcting refractive error separately with (i) trial lenses placed at the anterior focal point (constant RIS in mm for all participants), and (ii) contact lenses (RIS increases in line with eye length). Temporal summation was investigated in a similar cohort (24 myopes, mean: -4.65DS, range: -1.00DS, -11.25DS; 21 controls, mean: +0.87DS, range -0.25D, +2.00D) by measuring achromatic contrast thresholds for six stimuli varying in duration (1.1 – 187.8ms, 0.43° diameter). RIS was kept constant. The upper limit of complete summation ('Ricco's Area' (RA) and 'Critical Duration' (CD) for spatial and temporal summation respectively) was estimated from the data with iterative two-phase regression analysis. Retinal temporal summation was also measured objectively using electrophysiology and analysing how the amplitude of response changed for stimuli of constant energy (3cd/m^2) but varying duration (0.5-100ms).

Results: With spectacle correction, RA was significantly larger (p=0.02, Mann Whitney U-test) in the myopes compared to controls (myopes median: -0.92 log deg^2, IQR: -1.10, -0.78; controls median: -1.14 log deg^2, IQR: -1.29, -1.07). However, for contact lens correction, there was no significant difference in RA (p=0.44) between groups (myopes median: -1.19 log deg^2, IQR: -1.28, -0.96; controls median: -1.14 log deg^2, IQR: -1.24, -0.87). There was also no significant difference in CD between groups measured psychophysically (myopes median: 44.3ms, IQR: 26.5, 51.2; controls median: 41.6ms, IQR: 27.3, 48.5), nor objectively with the electroretinogram.

Conclusions: The area of complete spatial summation was altered in myopia when differences in projected RIS were accounted for, likely a compensation for reduced cell density secondary to axial elongation. However, when RIS was allowed to increase in line with axial length, there was no measurable difference in spatial visual function in myopia. In addition, temporal

summation was unchanged in myopia. This contrasts to glaucoma, where both spatial and temporal summation are altered. Taken together, these results suggest that reduced visual function in myopia is due to reduced cell density, rather than dysfunction in the cells themselves.

SA39

Associations between myopia risk polymorphisms and retinal electrophysiology

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Myopia, most often caused by elongation of the eye, is a leading, and increasing, cause of vision impairment globally. Existing evidence indicates retinal signalling has a key role in driving ocular growth. Genome-wide association studies have identified many genetic polymorphisms associated with myopia in the general population. Several of these are near genes expressed in the retina and involved in retinal physiology. The common variant most strongly associated with myopia is at locus rs524952 near the *GJD2* gene, which encodes the Connexin-36 protein, forming retinal gap junctions. The electroretinogram (ERG) represents the electrical response of the retina to light stimuli, and it can be recorded non-invasively from the living human eye. Here, we present findings from our studies investigating associations between myopia risk polymorphisms and ERG parameters. TwinsUK comprises several thousand adult twins who have volunteered to participate in research studies based at St Thomas' Hospital in London. Initially, dark-adapted and light-adapted ERGs were recorded in response to international standard and experimental stimulus protocols in over 200 twins, following pharmacological pupil dilation (1.0% tropicamide supplemented in most cases with 2.5% phenylephrine), using a conductive fibre electrode placed in the lower conjunctival fornix. Stimuli were delivered using the Colordome (Diagnosys UK, Cambridge, UK). In genotyped individuals (n=186), we specifically investigated associations between allelic dosage at rs524952 and ERG parameters (amplitudes and peak times of a-waves, b-waves and 30 Hz flicker responses), using a mixed linear model, adjusting for age, sex and familial relatedness. We found significant associations with parameters relating to cone-driven retinal signals. Taken together with findings in patients with selective loss of post-receptoral signals, we found evidence of a specific association with cone-driven OFF bipolar cell signals. Subsequently, we analysed ERG recordings from over 1000 twins, made with a portable device (RETeval system, LKC technologies, Gaithersburg, MD) and using skin electrodes. These were responses to 30 Hz flickering stimuli delivered through natural pupils, but with stimulus and background strength adjusted according to pupil diameter to deliver retinal illuminance equivalent to international standards. In genotyped individuals (n=895), we explored associations between 334 known myopia-risk loci and ERG flicker peak times. Although no association achieved statistical significance after correction for multiple testing, one of the top loci attaining nominal significance was rs13268738, within the *CNGB3* gene (which encodes a subunit of the cyclic nucleotide-gated channel in the outer segments of cone photoreceptors). This specific locus was then examined in the group of participants who had undergone mydriatic recordings with the conductive fibre electrode: allelic dosage was found to be significantly associated with flicker peak times in this groups also, thus replicating the association found in the larger group. Overall, our findings highlight possible pathways through which these particular myopia risk loci might be acting, supporting a role for alterations in retinal cone-driven signalling in myopia development.

SA40

The landscape of genetic variants conferring susceptibility to myopia in the general population

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Genome-wide association studies have transformed our understanding of the pathophysiology of common diseases in the last 15 years, and risk prediction through polygenic risk scores is now a reality. For myopia, the first two common genetic variants were described in 2010, and within ten years almost 450 genetic loci were identified, with the power obtained through increasingly large studies, the most recent including over half a million participants. Refractive error, and myopia in particular, is not dissimilar to height genetically, in that the trait is highly heritable but that risk is transmitted through many risk variants all of relatively small effect size. In keeping with our understanding of the physiology of emmetropisation being driven by the retina sensing defocus and regulating eye growth, stimulating axial elongation where there is hyperopic defocus and stopping growth when emmetropia or myopia is reached, many genes involved in retinal signalling have been associated with myopia. Where mutations in genes cause often devastating retinal dysfunction and impair vision, the common variants identified may cause (or are markers of) relatively minor functional changes, resulting in disturbance of the normal physiological processes. In common with many other GWAS studies, there is still a limited knowledge of the mechanisms involved. One of the commonest risk variants of strongest effect is on chromosome 15, near the *GJD2* gene which encodes the Connexin-36 protein, forming retinal gap junctions. Other examples include *KCNQ5, GRIA4, CACNA1D, RGR, RDH5, GNB3* and *RORB*. Bioinformatic tools (eg DEPICT) show the most significant gene sets associated with myopia are ‘abnormal photoreceptor inner segment morphology’, ‘thin retinal outer nuclear layer’, ‘detection of light stimulus’ and ‘nonmotile primary cilium’. Other genes that appear related to refractive error include those associated with circadian rhythm and pigmentation.

Hysi PG et al (2020) Nat Genet 52:401-407 Tedja MS et al (2018) Nat Genet 50:834-838

SA41

Structural insights into the mechanism of glycine transport and inhibition

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Glycine transporter GlyT1 (encoded by *SCL6A9*) is the main regulator of neuronal excitation and inhibition mediated by neurotransmitter glycine in the brain. Prolonging glycinergic signalling through selective inhibition of GlyT1 has been pursued extensively over the past two decades as a key strategy for the treatment of a broad range of neurological/psychiatric disorders including schizophrenia. GlyT1 inhibitors achieve antipsychotic and pro-cognitive effects against many symptoms of schizophrenia, however a successful drug candidate has to come. To elucidate structure-based mechanisms for inhibition and transport in GlyT1, we have investigated its complexes with a benzoylpiperazine chemotype inhibitor and substrate glycine. Using an inhibition state-specific sybody and a serial synchrotron crystallography (SSX) approach, we have determined the structure of GlyT1 at 3.4 Å resolution to reveal the selective inhibitor-bound state, adopting an inward-open conformation. More recently, we have determined the cryo-electron microscopy (cryo-EM) structure of GlyT1 at 3.3 Å resolution showing the glycine-bound inward-facing occluded conformation. The data unveil a dual nature of non-competitive inhibitors of functional transport exhibiting also competitive binding to the substrate binding site of glycine. The results provide detailed insight into the mechanism of glycine transport and reuptake inhibition and help re-evaluate efforts for the development of efficacious GlyT1 inhibitors.

SA42

The extended SLC Atlas: towards a unified view

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Background and aims: Transport of solutes across various biological membranes is essential to maintain cellular homeostasis and metabolism, and its dysfunction plays a pivotal role in the development of various diseases. The Solute Carrier (SLC) superfamily represents the largest and most diverse group of membrane transporter proteins, raising significant challenges in their identification, classification and annotation. Heterogeneity of SLC members also manifests at the level of protein structures, leading to several distinct structural folds among SLC transporters. Due to the absence of conserved sequence or structural signature motifs, we hypothesized that yet unidentified SLC transporters could exist in the human genome. In our work, we have undertaken a systematic meta-analysis of available data and literature in order to discover SLC-like proteins not yet in the official nomenclature. Contrary to similar analyses, we have strived to also find SLC-like proteins that are markedly dissimilar in sequence to the currently annotated ones, as well as use available structural information to define SLC superfamilies. A complete view of the human SLC-ome will play an instrumental role in understanding human physiology and can potentially be exploited for therapeutic benefits.

Methods: As a basis of our analysis, the Transporter Classification Database (TCDB), Protein families (Pfam), Uni-Prot, Protein Data Bank (PDB) databases have been used. Sequence similarity search was carried out using sequence profile hidden Markov-models (HMMs), using either models built by ourselves for individual TCDB protein families, or models obtained from Pfam.

Results: In order to perform a top-down search of SLC-like proteins, we have derived a set of eight criteria defining “SLC-likeness” in terms of properties that can be extracted from available databases. Manual curation of TCDB protein families and corresponding Pfam models was carried out based on the textual description of the families at the TCDB and Pfam web sites, respectively, in order to filter proteins and Pfam models that violate any of our SLC-likeness criteria. The remaining 166 protein families and 217 Pfam models were then used in sequence similarity searches against the proteomes of seven clinically relevant organisms, including human, rat, and mouse. The resulting 3669 proteins, including 520 from human, have subsequently been classified into families based on their pattern of similarity (fingerprint) to individual HMMs used in the search. Our analysis gave ~120 additional (“novel”), potentially SLC-like proteins compared to previously annotated SLCs, as well as ~40 additional protein families. Subsequent literature search on the found human proteins revealed that 53 of the “novel” SLC-like proteins could be assigned a small-molecule substrate.

Conclusions: The “newly” found transporters represent proteins that might have received less attention from the scientific community due to being missing from the official SLC nomenclature. In addition, several other putative SLC-like transporter proteins have been found. Subsequent analysis of structural homologs or predicted structures can identify further evolutionary relationships between the newly defined protein families. In summary, our results pave the way

to a more unified view of the complete cellular “SLC-ome”, essential for a thorough understanding of fundamental physiological and pathological processes.

Gyimesi G, Hediger MA. "Systematic in silico discovery of novel solute carrier-like proteins from proteomes." PLoS One. 2022;17: e0271062. doi:10.1371/journal.pone.0271062

SA43

Predicting the physiological function of SLC nutrient transporters in models of symbiosis

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Our current knowledge of solute carrier (SLC) transporter proteins comes from decades of detailed functional studies and more recent structural analyses. However, it remains problematic to accurately predict function of uncharacterised SLC proteins from across all kingdoms of life. Many animals have nutritional symbiotic partnerships with microbes which are intrinsically dependent on directional transport of nutrients between species. Such microbial symbionts can aid food digestion or synthesise nutrients missing from the host animal's diet. For example, sap feeding insects such as aphids, and blood feeding insects such as lice, have intracellular symbionts who provide their host insect with essential amino acids and vitamins. For these relationships to be maintained, specific transporters must function at multiple membranes in both species, yet little is known as to the identity and function of the proteins involved. We have used knowledge of bacterial and mammalian SLC transporters to accurately predict the function of aphid transporters involved in facilitating nutritional symbiosis. By doing so we are not only advancing knowledge of the molecular mechanisms central to a fundamental aspect of invertebrate pest biology but also of how an archetypal transporter binding pocket has evolved to produce a multitude of protein functions.

SA44

The Role of SLC transporters in Host-Tumour Metabolic Interactions

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Cancer is a systemic disease that is associated with host metabolic changes, including obesity, diabetes, and cachexia/muscle wasting syndrome, each of which alters the host's metabolic and nutritional environment. Cancer cells actively acquire nutrients from the extracellular space to support their growth, but how they sense and respond to changes in systemic nutrient availability remains incompletely understood. To explore host-tumour metabolic and nutritional interactions, we use the fruit fly *Drosophila melanogaster* as a model system. Our studies have started to uncover how tumours modulate the expression of SLC transporters to respond to systemic metabolic changes. These findings provide insight into the mechanisms by which tumours adapt to changes in nutrient availability and offer a potential therapeutic strategy to target transporters for cancer treatment.