

SA01

Muscle mitochondrial dysfunction in astronauts during spaceflight. What we can learn from and about the space environment.

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We analyzed the muscle biopsies of two astronauts who spent six months on the International Space Station (ISS) using mass spectrometry-based proteomics, with the aim of assessing how spaceflight modifies the structure and function of human skeletal muscle. We quantified on average 3800 proteins per sample, and a total of over 7000 proteins, spanning seven orders of magnitude in intensity. The two astronauts trained onboard using a treadmill and an Advanced Resistive Exercise Device (aRED) to simulate the use of free weights in the absence of gravity. The astronaut A trained more intensively and could preserve 80% of his muscle fiber cross-sectional area, compared to only 50% of astronaut B. Comparing the biopsies taken pre-mission to those from the day of landing we revealed in both astronauts a dramatic decrease in the expression of mitochondrial proteins located in all main compartments of the organelle, particularly the inner mitochondrial membrane and the matrix. The correlation of the mitochondrial protein loss to the relative amount of physical exercise was less straightforward than for the preservation of muscle mass. Our results are in line with the emerging consensus that mitochondria are a hub of the impact of spaceflight in humans as well as in various model organisms that were stationed on the ISS. Our data show that the relationship between exercise, muscle growth and mitochondrial biogenesis works differently in the space environment. My talk will discuss reactive oxygen species as a possible direct cause of mitochondrial damage and propose a role of physical exercise in the induction of an antioxidant response which can partially safeguard skeletal muscle structure and function.

SA02

Extreme postprandial muscle growth in a sedentary ectotherm: Gut-derived humoral signals trigger a rapid postprandial anabolic response in the digesting Burmese python.

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While physical inactivity poses significant health risks for humans, certain animal species have evolved remarkable adaptations to thrive despite sedentary lifestyles. Among these, the Burmese python (*Python bivittatus*), a sit-and-wait predator renowned for their capacity to devour massive prey items, stands out for its ability to undergo extreme postprandial muscle growth, even in the absence of physical activity.

Recent findings from our laboratory reveals an unexpected increase in metabolism and protein synthesis in skeletal muscles already within the first few hours after feeding, even before the prey has been digested in the stomach. Using chemical inhibitors targeting the protein degradation systems, we elucidated that this early anabolic response occurs through increased breakdown of endogenous proteins, compensating for the absence of exogenous nutrients.

Our current investigation delves into the mechanisms underlying this metabolic transition. Through blood transfusion experiments, we demonstrate that fasting snakes receiving blood plasma from digesting counterparts, sampled in the pre-absorptive phase of digestion, exhibit elevated skeletal muscle protein synthesis. Furthermore, by sequentially obstructing and/or circumventing different segments of the gastrointestinal tract, we ascertain the pivotal role of the small intestines in the initial stimulation of muscle growth following feeding.

Collectively, our observations suggest that during early digestion, humoral signalling originating from the small intestines triggers the rapid increase in postprandial protein synthesis in the skeletal muscle tissue of the Burmese python. Our findings not only uncover fascinating insights into the Burmese python's unique adaptations but also hold promise for future clinical applications. By revealing the role of humoral signaling from the small intestines in stimulating muscle growth, our research suggests potential avenues for developing therapies to combat muscle wasting disorders in humans, offering hope for patients with conditions such as sarcopenia or cachexia.

SA03

Harnessing the gut microbiota to facilitate muscle protein synthesis during fasting hibernation.

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Hibernation is a mammalian adaptation to wintertime food scarcity. It is the net manifestation of multiple underlying traits, and the most important of these is torpor, a regulated depression of metabolism that reduces wintertime metabolic rate – and thus energy use – by up to 98% relative to basal summertime rates. This allows hibernators to forego eating and rely entirely on stored fat for multiple months at a time, effectively solving the food scarcity problem. However, in the process, hibernation presents the animal with an array of “collateral” challenges to which they have needed to evolve resilience. One of these challenges is the risk of muscle atrophy, which arises from the combined effects of fasting and inactivity that typify the hibernation season. It has been known for over 20 years that hibernating mammals are resistant to muscle atrophy, but the mechanisms underlying this resistance, including the cellular mechanisms of protein balance and the continued supply of nitrogen despite the lack of exogenous source, are still being resolved. Recently, we have shown that hibernating ground squirrels obtain nitrogen during the winter fast by salvaging the nitrogen atoms present in urea using a gut microbe-dependent process called urea nitrogen salvage (UNS). The microbially liberated urea nitrogen is subsequently absorbed and incorporated into the protein content of liver and skeletal muscle, a process that progressively increases from the summer active season to early winter, and then peaks in late winter just prior to spring emergence. We are now in the process of characterizing which proteins in the skeletal muscle and heart are synthesized with the aid of UNS. We are conducting this project in the context of possible countermeasure development for spaceflight-induced disuse atrophy, as it is known that humans are capable of using UNS under certain conditions. This suggests that the necessary machinery for UNS is in present in humans, and so the mechanisms by which hibernators optimize their machinery to maximize UNS late in the hibernation season may provide insight into how our own UNS machinery may be optimized. This could potentially benefit humans under a variety of muscle atrophying conditions, particularly those in which limited nitrogen supply is a factor.

Regan, M.D., Chiang, E., Liu, Y., Tonelli, M., Verdoorn, K.M., Gugel, S.R., Suen, G., Carey, H.V. and Assadi-Porter, F.M., 2022. Nitrogen recycling via gut symbionts increases in ground squirrels over the hibernation season. *Science*, 375(6579), pp.460-463.

SA06

Retroviral transposable elements and the pregnancy remodelling functions of human trophoblast

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Complications of pregnancy, such as recurrent pregnancy loss, pre-eclampsia, fetal growth restriction, and spontaneous preterm birth, affect ~20 % of human pregnancies, causing maternal and fetal morbidity and mortality. Although the molecular aetiology of these disorders is not well understood, they are thought to share a common pathogenesis, in insufficient uterine invasion by the placenta. Transposable elements (TEs) comprise 50 % of our genome and are increasingly recognised as important in human physiology and disease, contributing to genetic variation and species-specific gene expression patterns. Previously, we found that a subset of transposable elements that have been co-opted following previous viral infections, known as endogenous retroviruses (ERVs), exhibit regulatory potential in the human placenta. These ERVs feature active chromatin marks specifically in placenta, and were bound by transcription factors with key roles in placental development. These elements were almost all primate-specific, and altered placental gene expression. To test their function in more depth, we used CRISPR-Cas9 excisions to show that several ERVs act as enhancers for key genes in the human placenta, such as CSF1R, ENG and PSG5. Research is now underway to explore whether human genetic and epigenetic variation at ERVs may contribute to pregnancy complications, using samples of placenta from normal and complicated pregnancies, and human trophoblast organoids.

SA07

Multi-omic mapping of human myometrium and decidua to decipher spontaneous labour.

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Greater understanding of physiological mechanisms driving parturition is needed if we are to develop more effective therapies for managing preterm and post-term birth. The problem of preterm birth (defined as birth before completion of 37 weeks gestation) exemplifies why action needs to be taken; it affects ~10% of all live births worldwide, acts as the leading cause of mortality for children under 5 years old, and presents a substantial risk of serious ill-health for surviving babies throughout the lifecourse [1-2]. Preterm birth can occur either spontaneously or by clinical intervention; spontaneous preterm labour (sPTL) typically accounts for almost half of all preterm births [3]. There are currently no reliable therapies available for preventing sPTL [4] due to notable gaps in our understanding of maternal-fetal interactions involved in coordinating the onset of spontaneous labour (for both preterm and term pregnancies). There is consensus that the myometrial and decidual tissue layers of the uterus are important contributors to labour. Myometrium predominantly comprises smooth muscle, which produces the uterine contractions that are characteristic of labour. Decidua is the mucosal lining between the myometrium and placenta/fetal membranes, which is integral to maternal-fetal immunomodulation and inflammatory events during parturition.

This talk presents the feasibility phase of the Borne Uterine Mapping Project (BUMP), which utilises a collaborative multidisciplinary approach to establish robust experimental protocols using innovative molecular mapping technologies, with the aim to explore multi- cellular and tissue processes that underpin the process of human labour. We applied bulk approaches for RNA-seq and proteomics to profile five distinct uterine sites (Figure 1) using biopsies acquired from term pregnancies undergoing Caesarean section while either not in labour or in early/established labour (LREC # 10/H0801/45; n=8 per group); single cell (sc) RNA-seq, single nucleus (sn) RNA-seq, spatial transcriptomics and sc proteomics were also used for biopsies from a separate cohort of not in labour and established labour Caesarean sections (n=4 per group).

Data from bulk approaches to RNA-seq and proteomics demonstrated regional differences between the five uterine sites of interest, which has important implications for understanding co-ordination of uterine contractile function. However, far less differences in gene and protein expression were significantly detected for labour status, especially for early labour. This supported the need to utilise scRNA-seq, snRNA-seq, spatial transcriptomics and sc proteomics, which delineate changes in mRNA or protein abundance between different cell types that exist in heterogenous tissues. Comparisons of scRNA-seq and snRNA-seq data for the same tissue type (decidua parietalis) will be made, and we will examine how well snRNA-seq can identify different cell types and their labour-related transcriptomic changes for three uterine regions according to our findings so far. Preliminary spatial transcriptomics data from upper segment (sub-parietalis) biopsies will also be presented to highlight the value of visualising changes in intact tissues for exploring how neighbouring cell types coordinate to mediate labour. Finally, contributions of these

data to our understanding of how the timing of birth is regulated will be discussed, and we will present plans to proceed with the analysis of samples from preterm pregnancies.

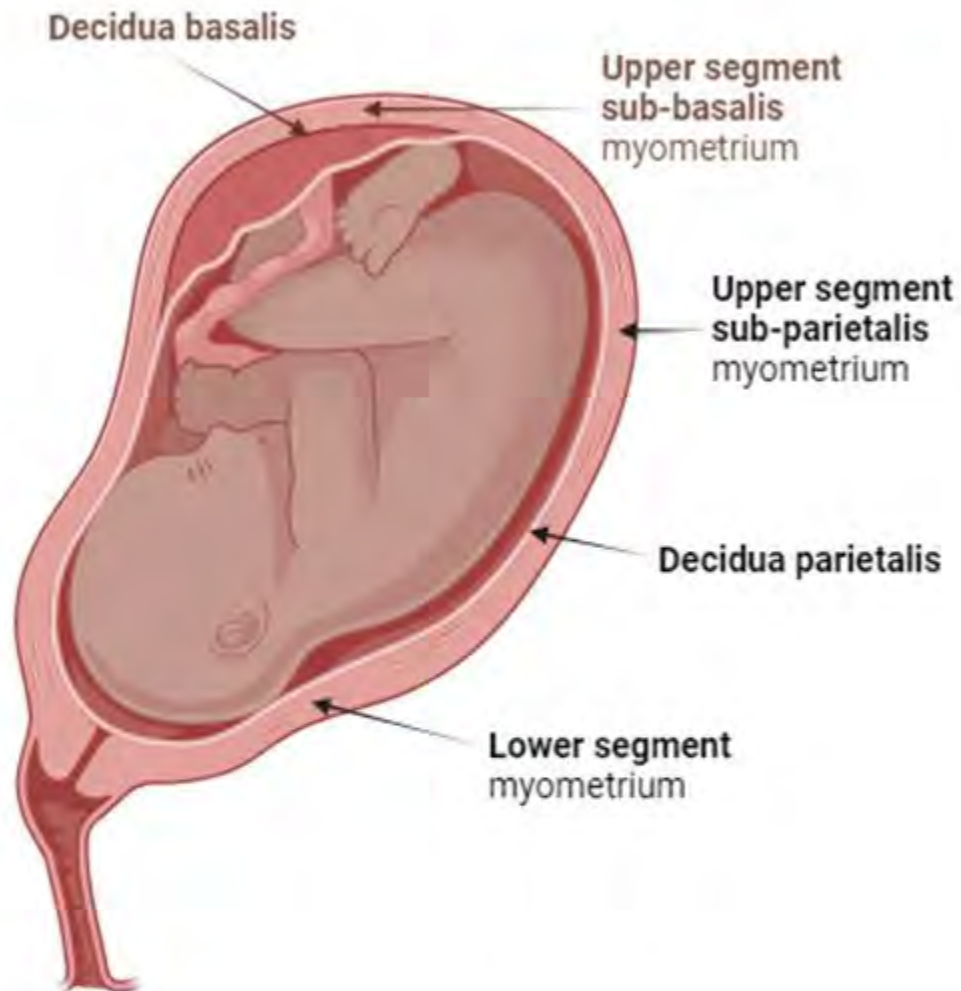


Figure 1. Uterine regions of interest for the Borne Uterine Mapping Project (BUMP). Created in Biorender.com

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SA08

The importance of developing a sense of student belonging for success and progression at university

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The graduate attributes and skills that we try to foster in our biomedical science and physiology-based programmes provide a set of skills, behaviours and values that we hope students will develop by connecting with the culture, curriculum and community of the university at which they study. The development of these attributes will rely to some extent on them feeling like they belong to a community and having a strong sense of belonging.

We will present and discuss the findings from student surveys on transition and sense of belonging that we have carried out for several years at the University of Birmingham. Data from our Biomedical Science students indicates that their sense of belonging increased as students progressed through the programme. This sense of belonging became less dependent on staff-student engagement as students progressed through their studies and alternative support structures such as having meaningful and strong networks became more important.

In year 1 students there was a high correlation between having a strong sense of belonging and having 1 or 2 close friends, students engaging with extra-curricular activities and having at least one staff member knowing their name. Students indicated that merely mixing students in large group meetings was not sufficient to develop a sense of belonging, but the opportunity to connect with like-minded students as a result of those meetings or through extracurricular engagement was reported to be more effective. This indicates the importance of meaningful interactions between students. If these opportunities are to be programme-based then staff need to engage with students through co-creation of activities.

First year students transitioning into the university showed a high level of interest in engaging with extracurricular activities. However, their active involvement did not match this desire to engage. Focus group discussions indicated the greatest barrier to engagement with extracurricular activities was student perception that they did not have enough time given the expectations and requirements of an academically challenging programme. There are, however, likely to be other contributing factors such as cost and part-time working. These results may link in some way to awarding and employability gaps.

We will facilitate an open discussion inviting ideas and examples of best practice on how to address the following questions:

How can we increase opportunities and communicate the importance of extracurricular activities for the development of graduate attributes in students?

How can we provide more opportunities for students to increase their networks and sense of community through meaningful activities?

Our data indicated that a high sense of belonging correlated well with students feeling more motivated to study, being more likely to form study groups, and being more likely to prepare for and participate in teaching sessions. This supports the literature which indicates an association between a sense of belonging and academic success, motivation, and self-belief (Freeman, Anderman and Jensen, 2007).

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<https://doi.org/10.3200/JEXE.75.3.203-220>

SA09

Enhancing belonging with programme-level synoptic assessment

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Students across several programmes at the University of Leeds (Biomedical Sciences, Neuroscience, and Pharmacology) share a substantial amount of teaching during first year. Focus groups were carried out with current students and the importance of belonging at the programme-level was highlighted, which was largely felt to be missing due to large-cohort shared teaching. As part of a period of educational transformation at Leeds, synoptic assessment was introduced within the school at Year 1. This was done in part to encourage students to transition from a modular view of learning to a more integrated approach, but also to encourage students to see the relevance of content to their programme within large-cohort shared modules.

In Semester 1, students complete synoptic assessment pieces in their broad discipline area. During Semester 2, all students engage with the same teaching material and formative problem-based application of knowledge short answer questions (SAQ's) during active learning workshops, followed by summative individual SAQ's. Each assessment piece contains "core knowledge" questions for students on all programmes, followed by programme-specific questions. To support programme-level assessment, programme meetings and community events were also implemented.

Reflections will be presented on some of the opportunities and challenges that have been encountered in implementing programme-level synoptic assessment across multiple modules of teaching, one of which is delivered outside of the school. The impact of this type of assessment is currently being assessed and initial findings will be shared as to whether it plays a role in encouraging student belonging to a programme of study.

SA10

The 'what' and 'why' of undergraduate physiology - student motivations for specialising in the science of life

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A students' sense of belonging is known to influence academic engagement, achievement and retention. At Newcastle, our large Biomedical and Biomolecular Sciences (BBS) cohort is split across five subjects but dominated yearly by Biomedical Sciences (BMS, ~200-250/360 students), with far fewer students opting to study Physiological Sciences. Teaching group size is known to influence students' ability to build meaningful relationships with peers and staff, influencing their sense of belonging, so the purpose of this project was to investigate BBS students' sense of belonging and whether it was influenced by or determined subject choice.

A survey sent to registered BBS students received 256 responses (25% response rate). The respondent population was 70% female, 85% aged 18-21, 77% Caucasian and 88% Home/EU students which broadly reflects the overall BBS student body. The data revealed little difference in overall sense of belonging (using a 10-point scale) by gender identity and fee status, but a decrease in sense of belonging as students advance through their BSc degree (6.6 vs 6.0 in year 1 vs year 3) and notable differences between degree programme with the large BMS cohort scoring lowest (6.1) and Physiological Sciences highest (6.6). Interestingly, 4th year MSci (7.1) and placement year/study abroad students (7.5) reported the highest overall sense of belonging.

Focus groups and further analyses of survey data are ongoing and the preliminary findings of these will be presented with a view to identify approaches to target and improve student engagement and sense of belonging across diverse student cohorts, and elicit ways to enhance student understanding of degree choice.

SA11

Focusing on belonging in curriculum design

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Community and student engagement is a global sector wide problem. There is a growing area of research within student education about sense of belonging and increased motivation for learning. Sense of belonging is one of the most significant factors in students' success and retention in higher education. It is something highly valued but rather hard to quantify. Despite its clear importance, many students report not feeling part of a community, in the UK, is reflected in low National Student Survey (NSS) scores for questions related to belonging sector wide.

At Leeds, recently the year 1 UG curriculum has been re-designed with the sense of belonging as a focus. This includes enhanced contact time, but still utilising a flipped classroom approach to teaching. A scaffolded approach to training students to engage with team work along with activities with autonomy of choice of the topic area studied within the team. This has been shown to support the building of a community within peers on their course.

A synoptic programme level assessment approach was also adapted which included reflective writing about both academic on course experiences but also extra curricula activities and part time work. This enabled students to link their whole university journey together to identify the skills they have identified and practiced throughout their first year of study.

Data will be discussed to support whether different activities within the undergraduate curriculum can support students to feel part of a community and therefore engaging more with their studies leading to higher student success rates.

SA12

Bidirectional metabokine and lipokine-mediated adipose tissue – skeletal muscle crosstalk during browning of adipose tissue

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Brown adipose tissue (BAT) regulates body temperature through non-shivering thermogenesis. Browning, the induction of a brown adipose-like phenotype in white adipose tissue (WAT), increases β -oxidation, mitochondrial biogenesis and thermogenesis(1). Brown adipocyte-like cells (beige adipocytes) are interspersed within WAT, and alter systemic energy balance with anti-obesity effects. In humans, BAT quantity correlates with decreased risk of diabetes and cardiovascular disease. However, BAT and beige adipose tissue can affect systemic energy balance independently of thermogenesis. Brown and beige adipose tissue thermogenesis leads to propagation of thermogenesis in surrounding and distal WAT and increases fat oxidation in skeletal muscle. This suggests brown/beige adipocytes influence systemic metabolism through interorgan signals in the adipocyte secretome. The nature of these signals is incompletely defined.

Metabolites were considered intermediates or end-products of metabolism, passive participants changed by metabolic processes. There is emerging evidence of metabolites, which function to mediate cellular signalling and interorgan crosstalk, regulating local metabolism and systemic physiology. These signals have been termed metabokines. Developing contemporaneously with the new appreciation of the complexity of tissue-crosstalk mediated by metabolites is an understanding that bioactive lipids also have a key role in the communication between cells, organs and tissues. These bioactive lipids have been termed lipokines. There is impetus to uncover novel metabokine signalling axes to understand how these are perturbed in metabolic diseases and determine their utility as therapeutic targets.

Our research has defined discrete brown/beige adipose tissue-derived metabokine and lipokine-mediated signalling axes that drive enhanced fatty acid oxidation in skeletal muscle. Reciprocally, we have identified an exercise-induced skeletal muscle-derived metabokine that drives adipose tissue browning. Here we will provide an overview of our research identifying novel metabokine signals:

1) Monocarboxylic acids secreted from thermogenic adipose tissue, which induce a thermogenic phenotype in white adipose tissue and oxidative energy metabolism in skeletal muscle to reduce adiposity, increase energy expenditure and improve glucose and insulin homeostasis in models of obesity and diabetes.

2) A lysophosphatidylcholine that functions as a BAT/beige adipocyte interorgan lipokine increasing fat oxidation in muscle and adipose tissue to increase systemic energy expenditure with anti-obesity effects

3) A bioactive non-protein β -aminoacid secreted from skeletal muscle during exercise, which induces adipose tissue thermogenesis and liver β -oxidation.

Our discoveries further our understanding of metabokine and lipokine mediated adipose tissue – skeletal muscle crosstalk, and the potential therapeutic role that these metabokines/lipokines may have to treat obesity and related cardiometabolic diseases.

SA13

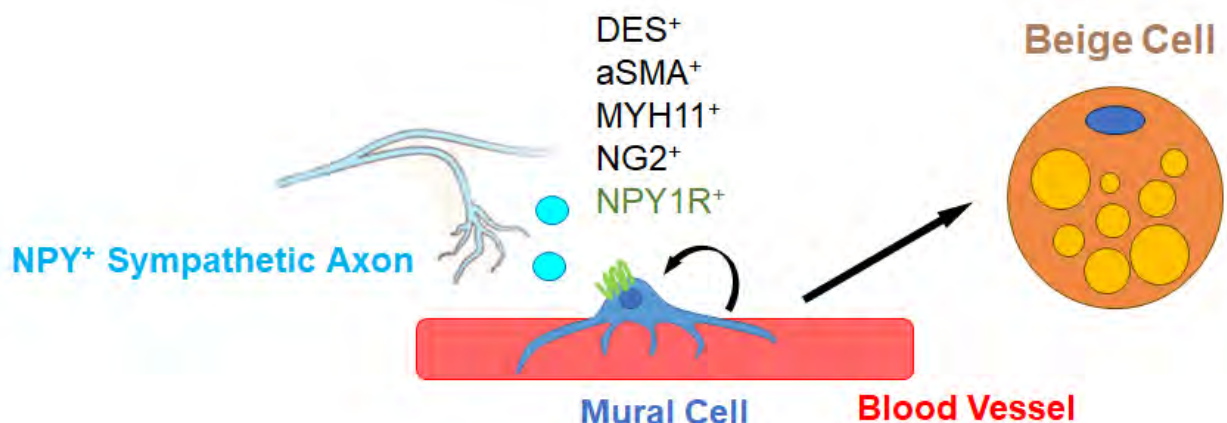
Sympathetic neuron-derived NPY protects from obesity by sustaining the mural progenitors of thermogenic adipocytes.

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Neuropeptide Y (NPY) is secreted by sympathetic nerves, but its direct impact on thermogenic adipocytes is unknown. Here we uncover the mechanism by which peripheral NPY protects from obesity. Our imaging of cleared murine brown and white adipose tissue (BAT and WAT) established that NPY+ sympathetic axons are only a minority that mostly maps to the peri-vasculature; our analysis of single-cell RNA-sequencing datasets identifies mural cells as the main NPY-responsive cells in adipose tissues. We show that NPY sustains mural cells, which are known to be a source of beige cells in both BAT and WAT and that NPY facilitates the differentiation to thermogenic adipocytes. We found that diet-induced obesity leads to neuropathy of NPY+ axons and concomitant depletion of the mural cell pool of beige fat progenitors. This defect is replicated in conditional knockout (cKO) mice with NPY specifically abrogated from sympathetic neurons. These cKO mice have whitened BAT with reduced thermogenic ability and lower energy expenditure even before the onset of obesity; they develop adult-onset obesity on a regular chow diet and are more susceptible to diet-induced obesity without increasing food consumption. Our results indicate that relative to central NPY, peripheral NPY produced by the sympathetic nerves has the opposite effect on body weight homeostasis by sustaining the proliferation of the mural cell progenitors of thermogenic adipocytes.



SA14

CIRCADIAN TRANSCRIPTOME OSCILLATIONS IN HUMAN ADIPOSE TISSUE DEPEND ON NAPPING STATUS AND LINK TO METABOLIC AND INFLAMMATORY PATHWAYS

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Intro. Napping is a common habit in many countries, but the impact of napping on general health is still unclear. Studies about the chronic effects of napping on obesity are contradictory, and the molecular link between napping and metabolic alterations has yet to be studied. Studies in animals and humans have shown that several genes expressed in adipose tissue (AT) follow a circadian rhythmic pattern, and disruption of circadian rhythms leads to obesity and metabolic disorders.

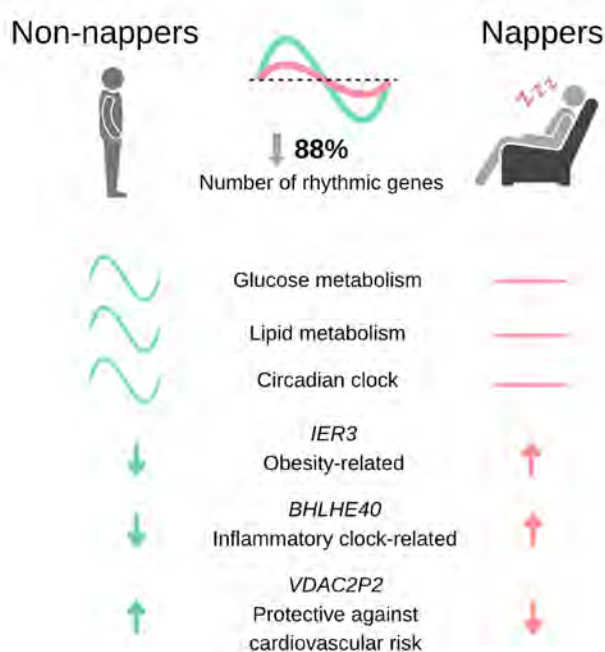
Aim. We aim to identify molecular mechanisms in AT that may connect napping and abdominal obesity. We propose that napping may induce, in AT, changes in the rhythmicity in those genes involved in energy metabolism, inflammation, and adipogenesis.

Method. We analyzed the circadian rhythmicity and global expression of genes expressed in 24-h cultured AT explants in 17 subjects with severe obesity, to assess differences between nappers (n=8) (long nappers, nap duration=1.18±0.7, mean±SD) and non-nappers (n=9). We extracted the RNA repeatedly across 24h from cultured AT explants of habitual nappers and non-nappers and performed RNA sequencing. Circadian rhythms were analyzed using 6 consecutive time points during 24 hours. Biostatistical and bioinformatic analyses were carried out in R. This work is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and procured in line with World Health Organization Guiding Principles on Human Cell, Tissue and Organ Transplantation.

Results. RNAseq analysis showed that 28.31% of the total genes analyzed in the total population displayed significant rhythms both by cosinor and non-parametric analyses. We further divided the total population into nappers and non-nappers to answer our main aim. In nappers, there was a loss of rhythmicity in 88% of genes that showed circadian rhythmicity among non-nappers (among non-nappers, 2666 genes were rhythmic, while among nappers, only 321 genes showed circadian rhythmicity). We observed a reduction in rhythm amplitudes of 29% in nappers and identified cholecystokinin (CCK) as the gene with the strongest decrease in amplitude in nappers. We also

found significant phase differences from a coherent unimodal acrophase in non-nappers towards a scattered and bimodal acrophase in nappers. *CTNNAL1* and *CHUK* were two relevant genes with significant delay in acrophase. Further pathway enrichment analysis showed that those genes that lost rhythmicity with napping were mainly involved in pathways of glucose and lipid metabolism, as well as of the circadian clock. Additionally, we found differential global gene expression between nappers and non-nappers, with 34 genes down- and 32 genes up-regulated in nappers. The top-up-regulated gene (*IER3*), another gene highly up-regulated (*BHLHE40*, a clock-related gene), and top-down-regulated pseudogene (*VDAC2P2*) in nappers have been previously shown to be involved in inflammation.

Conclusion. These findings not only resolve conflicting evidence on napping's chronic effects on obesity but also offer crucial insights into molecular mechanisms, shaping our understanding of napping's role in metabolic disorders. Since napping during the day, especially long napping, may alter the AT circadian rhythm and this may influence obesity, we would recommend not taking long naps, based on our previous results.



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SA15

Esr1+ hypothalamic-habenula neurons shape aversive states

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Excitatory projections from the lateral hypothalamic area (LHA) to the lateral habenula (LHb) drive aversive responses. We used patch-sequencing (Patch-seq) guided multimodal classification to define the structural and functional heterogeneity of the LHA-LHb pathway. Our classification identified six glutamatergic neuron types with unique electrophysiological properties, molecular profiles and projection patterns. We found that genetically defined LHA-LHb neurons signal distinct aspects of emotional or naturalistic behaviors, such as estrogen receptor 1-expressing (Esr1⁺) LHA-LHb neurons induce aversion, whereas neuropeptide Y-expressing (Npy⁺) LHA-LHb neurons control rearing behavior. Repeated optogenetic drive of Esr1⁺ LHA-LHb neurons induces a behaviorally persistent aversive state, and large-scale recordings showed a region-specific neural representation of the aversive signals in the prelimbic region of the prefrontal cortex. We further found that exposure to unpredictable mild shocks induced a sex-specific sensitivity to develop a stress state in female mice, which was associated with a specific shift in the intrinsic properties of bursting-type Esr1⁺ LHA-LHb neurons. In summary, we describe the diversity of LHA-LHb neuron types and provide evidence for the role of Esr1⁺ neurons in aversion and sexually dimorphic stress sensitivity.

SA16

The Forgotten Circulation: Reduced mesenteric venous capacitance in hypertensive rats is improved by decreasing sympathetic activity

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The mesenteric venous reservoir plays a vital role in mediating blood volume and/or pressure changes and is richly innervated by sympathetic nerves; however, the precise nature of venous sympathetic regulation and its role during hypertension remains unclear. We hypothesized that sympathetic drive to mesenteric veins in spontaneously hypertensive (SH) rats is raised, increasing mean circulatory filling pressure (MCFP), and impairing mesenteric capacitance.

Arterial pressure, central venous pressure, mesenteric arterial and venous blood flow were measured simultaneously in conscious male Wistar and SH rats. MCFP was assessed using an intra-atrial balloon. Hemodynamic responses to volume changes ($\pm 20\%$) were measured before and after ganglionic blockade and carotid body denervation (CBD). Sympathetic venoconstrictor activity was measured in situ.

MCFP in vivo (10.8 ± 1.6 vs 8.0 ± 2.1 mmHg; $P=0.0005$) and sympathetic venoconstrictor drive in situ (18 ± 1 vs 10 ± 2 μ V; $P<0.0001$) were higher in SH rats; MCFP decreased in SH rats after hexamethonium and CBD (7.6 ± 1.4 ; $P<0.0001$ and 8.5 ± 1.0 mmHg; $P=0.0045$). During volume changes, arterial pressure remained stable. With blood loss, net efflux of blood from the mesenteric bed was measured in both strains. However, during volume infusion, we observed net influx in Wistar ($+2.3 \pm 2.6$ ml/min) but efflux in SH rats (-1.0 ± 1.0 ml/min; $P=0.0032$); this counterintuitive efflux was abolished by hexamethonium and CBD ($+0.3 \pm 1.7$ and 0.5 ± 1.6 ml/min, respectively).

In SH rats, excessive sympathetic venoconstriction elevates MCFP and reduces capacitance, impairing volume buffering by mesenteric veins. We propose selective targeting of mesenteric veins through sympathetic drive reduction as a novel therapeutic opportunity for hypertension.

SA17

The elusive but large role of the venous system in vasovagal syncope and orthostatic hypotension

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Vasovagal syncope (VVS) and classical orthostatic hypotension (cOH) are very common causes of syncope and orthostatic intolerance. VVS causes syncope at least once in one third of all people and cOH occurs with or without syncope in 25-30% of the elderly.

Both can be evoked with a 'tilt test' (TT). Applying continuous non-invasive blood pressure recordings and analysis software ('Modelflow') yield estimates of the three determinants of Mean Arterial Pressure (MAP), i.e., heart rate (HR), stroke volume (SV) and total peripheral resistance (TPR).

VVS is most often triggered by pain, fear ('emotional VVS') or just standing ('orthostatic VVS'); TT studies of the latter type of VVS showed that the first abnormality is a gradual decrease of SV, explained through by gradual venous pooling in the splanchnic and probably muscle venous beds. This causes a moderate decrease of MAP, which, if not countered by sitting or lying, can in turn evoke a sudden calamitous decrease of HR causing an ever faster decrease of MAP, ending in syncope. It is unclear how and why standing occasionally causes venous pooling that sets the VVS cascade in motion, functioning normally on other occasions.

cOH is by definition triggered by standing. The best understood mechanism is autonomic damage causing a triple defect. First, a failure of TPR to increase when upright causes an 'arterial leak'; second, a simultaneous decrease of SV again suggests venous pooling, presumably because of deficient venous vasoconstriction; third, a failure of HR to increase can cause very low upright MAP. The contribution of low SV, i.e., venous pooling to cOH is probably underestimated.

In VVS as well as cOH, the role of the venous system is implied by alterations on the arterial side of the circulation. Understanding the pathophysiology of VVS and cOH could benefit from tools that measure fluid distribution over venous beds and assess venous vasoconstriction.

SA18

Use it or lose it: the importance of contractile activity for maintenance of skeletal muscle mass and function in older age.

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Beyond their most commonly recognised roles in locomotion and postural support, skeletal muscles also serve as the central node for whole-body metabolic health, acting as, for example, the largest amino acid (AA) reservoir and glucose disposal site in the body. Alongside AA nutrition, muscle contraction in the form of exercise and/ or physical activity is well established to improve metabolic health and induce both favourable physiological adaptations (e.g., muscle mass gains, mitochondrial biogenesis) and secondary health benefits (e.g., improved insulin sensitivity). Indeed, nutrition x contractile interaction is the fundamental basis of muscle mass maintenance across the life course. In stark contrast, the absence of muscle contraction in the form of “disuse” (e.g. through simple inactivity, casting, immobilization, or bed rest) has the opposite effect causing muscle atrophy, functional declines, and metabolic impairment (e.g., muscle insulin resistance). In older adults, events leading to periods of disuse or reduced physical activity are relatively commonplace (i.e., after falls, surgery, or during illness), with the additional complication of an existent background of sarcopenia progression in these individuals. Despite it being largely accepted that the regulatory processes underpinning muscle mass and functional declines with both advancing age and as a result of disuse are complex and multifactorial, we are still some way from complete understanding; with phenomenon such as atrophy resistant versus atrophy susceptible (aRaS) muscles posing novel questions. This presentation will i) outline the importance and underlying mechanisms of skeletal muscle mass maintenance for older adults, ii) highlight the rapidity of skeletal muscle disuse atrophy in both healthy and clinical cohorts, iii) address the observation of aRaS muscles, and iv) finally, provide early-data from studies looking to develop intervention strategies to mitigate losses of muscle mass and function in older surgical patients.

SA19

Fusion of myofibre branches is a physiological feature of healthy human skeletal muscle regeneration

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Background: The occurrence of hyperplasia, through myofibre splitting, remains a widely debated phenomenon. Structural alterations and fibre typing of skeletal muscle fibres, as seen during regeneration and in certain muscle diseases, can be challenging to interpret. Neuromuscular electrical stimulation can induce myofibre necrosis followed by changes in spatial and temporal cellular processes. Thirty days following electrical stimulation, remnants of regeneration can be seen in the myofibre and its basement membrane as the presence of small myofibres and encroachment of sarcolemma and basement membrane (suggestive of myofibre branching/splitting). The purpose of this study was to investigate myofibre branching and fibre type in a systematic manner in human skeletal muscle undergoing adult regenerative myogenesis.

Methods: Electrical stimulation was used to induce myofibre necrosis to the vastus lateralis muscle of one leg in 5 young healthy males. Muscle tissue samples were collected from the stimulated leg 30 days later and from the control leg for comparison. Biopsies were sectioned and stained for dystrophin and laminin to label the sarcolemma and basement membrane, respectively, as well as ATPase, and antibodies against types I and II myosin, and embryonic and neonatal myosin. Myofibre branches were followed through 22 serial Sects. (264 µm). Single fibres and tissue blocks were examined by confocal and electron microscopy, respectively.

Results: Regular branching of small myofibre segments was observed (median length 144 µm), most of which were observed to fuse further along the parent fibre. Central nuclei were frequently observed at the point of branching/ fusion. The branch commonly presented with a more immature profile (nestin + , neonatal myosin + , disorganised myofilaments) than the parent myofibre, together suggesting fusion of the branch, rather than splitting. Of the 210 regenerating muscle fibres evaluated, 99.5% were type II fibres, indicating preferential damage to type II fibres with our protocol. Furthermore, these fibres demonstrated 7 different stages of “fibre-type” profiles.

Conclusions: By studying the regenerating tissue 30 days later with a range of microscopy techniques, we find that so-called myofibre branching or splitting is more likely to be fusion of myotubes and is therefore explained by incomplete regeneration after a necrosis-inducing event.

SA20

Acute effects of exercise on brain metabolites and cognition in older adults

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The health benefits of physical activity are widespread across the human body – including the brain. Particularly in older adults, physical activity has been shown to promote cognitive function, protect brain structure against expected age-related declines, and potentially even reduce the risk, or delay the onset, of dementia. Using *in vivo* neuroimaging methods, the last decade has seen significant developments in our understanding of the mechanisms behind these effects. Using proton Magnetic Resonance Spectroscopy (1H-MRS), metabolites which are more commonly found in glia than in neurons, such as myo-inositol (mIns), total creatine (tCr) and total choline (tCho), have been shown to be increased in older adults. Overactive glial cells - as observed in some brain diseases - can result in chronic neuroinflammation. Importantly, the same metabolites have been reported to negatively correlate with performance on a working memory task, suggesting a potential mechanistic link between age-related changes in brain metabolites and cognitive function. The current study will test whether exercise modulates these markers of neuroinflammation in older adults and, crucially, if these changes explain exercise-induced cognitive improvements. To examine the effects of a single exercise session on brain metabolite concentrations and working memory function, forty-eight older adults (65 – 75 years old; 50% women) will undergo a working memory test and 1H-MRS imaging at 7 Tesla before and after a cycling exercise. Exercise protocols are individualized to each participant, based on their performance on a VO2 max fitness test. In addition, participants will attend a control session, where they will undergo the same working memory test and brain scans before and after remaining seated. Data collection is currently underway. If exercise-induced metabolite changes are associated with cognitive changes, then these findings will contribute to our understanding of the underlying neural mechanisms of exercise-induced brain benefits in ageing.

SA21

Mind the Brain: Powering your brain through exercise and diet

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Lifestyle factors, including exercise and diet, is offering a promising solution for age-related negative effects on brain health, with a potential to postpone the onset of neurodegenerative diseases. The results, however, are muffled with large individual difference in the effects, and it appears as if one-size does not fit all. In this talk I will provide an overview about our current understanding about the link between exercise- and dietary-induced brain plasticity in aging. I will also discuss potential reasons why some individuals gain from these lifestyle interventions and why some individuals appear to have little room for brain plasticity. I believe that understanding these factors is the necessary next step to harvest the full potential of lifestyle factors for brain health.

SA22

Contribution(s) of the permeability transition pore to the pathophysiology of Vici syndrome, a severe multisystem disorder of childhood.

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The mitochondrial permeability transition pore (mPTP) seems to be a critical final common path to cell injury and death in multiple disease states. Discovered in the 1970's by Hunter and Haworth, the critical trigger for pore opening is an excess of calcium in the mitochondrial matrix, while oxidative or nitrosative stress lower the threshold for calcium induced pore opening. Once the large conductance pore in the inner mitochondrial membrane has opened, the membrane potential will collapse causing depletion of ATP, mitochondria swell and – if the whole population of mitochondria in a cell undergo permeability transition – cell death. Critically, pore opening can be limited pharmacologically, making the pore a potential therapeutic target for multiple diseases (for a recent review, see (1)).

I will talk about a disease in which a pathophysiological cascade seems to involve opening of the mPTP probably in a subset of mitochondria with more complex but critical consequences. Vici syndrome is an apparently rare devastating early onset multisystem disorder caused by mutations of a protein known as EPG5, which plays a key role in autophagy, mediating fusion specificity of the autophagosome and lysosome. Almost every system is involved in Vici syndrome but in addition to multiple developmental defects, neurological and neuromuscular features are most prominent. Affected children suffer from a progressive neurodegeneration that, in combination with severe cardiorespiratory involvement, is usually fatal by the age of 10 at the severe end of the disease spectrum.

In fibroblasts from children with EPG5 mutations, mitophagy is significantly impaired². We have found that, as a consequence, mitochondria are profoundly dysfunctional, with impaired respiration and reduced mitochondrial membrane potential. In exploring the impact of a calcium signal on mitochondrial function in these cells, we were surprised to find that mitochondrial calcium uptake was paradoxically significantly increased. This was attributable to downregulation of MICU1, the Ca²⁺ dependent gatekeeper of mitochondrial calcium uptake. As a consequence, mitochondria from these cells show an increased vulnerability to mPTP opening in response to normally innocuous calcium signals. This is followed by release of mtDNA into the cytosol which

activates the cGAS/STING pathway, initiating the innate inflammatory response. It seems probable that this response plays a significant role in shaping the disease presentation and progression, especially in relation to the neurodegenerative disorder. These data point to multiple steps at which therapeutic intervention might prove beneficial – pharmacological stimulation of mitophagy, reducing mitochondrial calcium overload, inhibition of the mPTP or inhibition of the cGAS/STING pathway.

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SA23

Electrophysiological Properties of the Inner Mitochondrial Membrane: Diversity of Mechanisms of Mitochondrial Dysfunction

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Mitochondrial permeability transition (mPT) is a phenomenon of a sudden increase in the permeability of the inner mitochondrial membrane (IMM). Patch-clamp experiments on isolated mitochondria identified the mPT mechanism as the Ca²⁺- and ROS-stimulated opening of a large, unspecific pore in the IMM, known as the permeability transition pore (mPTP). The opening of the mPTP disrupts normal mitochondrial function and has been implicated as the hallmark of mitochondria-driven cell death in many diseases, including ischemia-reperfusion injuries of organs.

Here, we applied advanced whole-mitoplast patch-clamp and *ex vivo* patch clamp approaches to investigate the still-understood mechanism of mitochondrial dysfunction associated with mPTP. The whole-mitoplast configuration allows us to observe mPTP currents at the level of the whole mitochondrion (Neginskaya & Pavlov, 2023), and *ex vivo* patch clamp detects the changes in IMM permeability induced by *in vivo* stress, contrasting with the stress applied to mitochondrial membranes after isolation (Niatsetsckaya et al., 2020). Patch-clamp findings were supported by holographic imaging (HI) in cultured MEF cells. HI detects changes in the refractive indexes of mitochondria upon mPTP opening, enabling the observation of mPTP within the living cell, independently of mitochondrial depolarization (Neginskaya, Morris, & Pavlov, 2022).

Whole-mitoplast recordings from isolated cardiac mitochondria demonstrated that Ca²⁺-induced currents were partially blocked by CSA (42%, n=12) and by ADP (100%, n=2), but not sensitive to bongkreikic acid (BA), an inhibitor of the Adenine Nucleotide Translocator. In contrast, the ROS-stimulated whole-mitoplast current was transiently blocked by BA (n=1), suggesting that the mechanisms of IMM permeability might vary depending on stress conditions.

Ex vivo patch-clamping of brain mitochondria isolated from neonatal (p10) mice exposed to hypoxia-ischemia brain injury detected elevated IMM permeability after 30 min of reperfusion (420±40 pS, n=54 vs. 250±40 pS in control, n=18; p=0.05, Kruskal-Wallis). This elevated IMM permeability did not exhibit the classic CSA-sensitive mPTP current behavior. Neuroprotective hypothermia applied during reperfusion attenuated the infarct volume of brain (n=15, 36.7±6.0% to 19.2±5.0%) and normalized the associated elevation of IMM permeability (420±40 pS, n=54 to 290±50 pS, n=30, p=0.03, Kruskal-Wallis). These results suggest the existence of a non-mPTP mechanism of mitochondrial damage contributing to the injury. Interestingly, hyperoxia during reperfusion resulted in the activation of the classical CSA-sensitive mPTP (80% of CSA-sensitive channels (n=10) vs. 14% (n=14) in normoxia, p=0.01, χ^2 test) and exacerbated post-ischemic injury.

Finally, HI demonstrated that mitochondrial Ca²⁺ overload induced by the ionophore ferutinin stimulates classic CSA-sensitive mPTP activation (Neginskaya et al., 2022), while ionomycin-

induced Ca^{2+} dysregulation triggered mitochondrial depolarization and swelling but not the mPTP opening (n=4). CSA did not inhibit ionomycin-induced mitochondrial depolarization and swelling (n=3), or subsequent cell death (n=2), but it abolished the effect of ferutinin.

Therefore, we have demonstrated the existence of at least two mechanisms: mPTP and non-mPTP mitochondrial dysfunction that might contribute to acute ischemia-reperfusion injuries, with each pathway's impact determined by the unique stress conditions.

Neginskaya, M. A., Morris, S. E., & Pavlov, E. V. (2022). Both ANT and ATPase are essential for mitochondrial permeability transition but not depolarization. *iScience*, 25(11), 105447. doi: 10.1016/j.isci.2022.105447 Neginskaya, M. A., & Pavlov, E. V. (2023). Investigation of Properties of the Mitochondrial Permeability Transition Pore Using Whole-Mitoplast Patch-Clamp Technique. *DNA Cell Biol.* doi: 10.1089/dna.2023.0171 Niatsetskaya, Z., Sosunov, S., Stepanova, A., Goldman, J., Galkin, A., Neginskaya, M., . . . Ten, V. (2020). Cyclophilin D-dependent oligodendrocyte mitochondrial ion leak contributes to neonatal white matter injury. *J Clin Invest*, 130(10), 5536-5550. doi: 10.1172/JCI133082

SA24

The interplay between mitochondrial calcium, permeability transition, and cell death in cardiac maladaptation to chronic stress

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The acute uptake of calcium (Ca^{2+}) into the mitochondrial matrix via the mitochondrial calcium uniporter is a key signal that enhances mitochondrial ATP production in parallel with stimuli that increase cytosolic Ca^{2+} signaling and cellular energy demand. This physiologic mitochondrial Ca^{2+} (mCa^{2+}) accumulation is critical for the heart to rapidly increase its workrate in response to sympathetic stimulation during exercise or fight-or-flight responses. In excess, though, acute mCa^{2+} loading triggers mitochondrial permeability transition (mPT) and necrotic cell death. Such mCa^{2+} -dependent mPT drives initial tissue injury and subsequent organ-level dysfunction in response to acute ischemic insults including myocardial infarction and stroke. However, the contribution of mCa^{2+} overload and mPT to more gradually-developing dysfunction in cardiovascular diseases featuring a sustained increase in cytosolic Ca^{2+} concentration remains poorly defined. This question is particularly relevant for forms of non-ischemic heart failure that develop over time following chronic elevations in cardiac afterload and neurohormonal stimulation. These pathologies feature progressive cardiomyocyte death that can compromise the contractile function of the heart. Understanding the relationship between mCa^{2+} accumulation, mPT, and cardiomyocyte dropout in the context of such chronic heart disease is complicated by the proposed homeostatic role of the mitochondrial permeability transition pore (mPTP) as a route for physiologic mCa^{2+} efflux that limits deleterious mCa^{2+} overload. This talk will highlight recent *in vivo* findings from genetic mouse models featuring manipulation of the mitochondrial calcium uniporter or the mCa^{2+} efflux machinery to modulate mCa^{2+} accumulation in experimental models of non-ischemic heart failure. It will also address the complex interplay between altered mCa^{2+} homeostasis, the proposed constituents of the mPTP, and cell death in the chronically-stressed heart.

SA25

Emerging Role of mPT in Driving Atrophy, Mitochondrial Dysfunction and Denervation in Skeletal Muscle with Aging and Disease

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Statement of the Problem: In contrast to the established role of mitochondrial permeability transition (mPT) in cardiac ischemia-reperfusion injury, neurodegeneration, and other tissue pathology, the role of mPT in skeletal muscle is less clear, notwithstanding some work in muscular dystrophies.

Methods: To address this gap, we used approaches in adult mouse single muscle fibers and isolated skeletal muscle mitochondria to establish the consequences of mPT in skeletal muscle. We induced mPT using Bz423, an agent that binds to the same site as cyclophilin D (CypD) on the oligomycin sensitivity conferring protein of the ATP synthase. We used inhibitors of mPT that worked through CypD (Alisporivir) or were CypD-independent (TR002, Isox63). We assessed mitochondrial ROS (mROS) generation using mitoSox, and inhibited mROS using mitoTEMPO. We assessed caspase 3 (Casp3) activation using a Casp3 FLICA assay, and inhibited Casp3 using a novel inhibitor (Ac-ATS0101-KE). We assessed mitochondrial respiration in isolated mouse skeletal muscle mitochondria. We evaluated the integrity of the acetylcholine receptor cluster (AChR) on single mouse muscle fibers by labeling with Alexa 488-conjugated α -bungarotoxin (α BG). To provide context to mPT in skeletal muscle, we performed a calcium retention capacity (CRC) in muscle bundles from elderly men and women, in mouse muscle fiber bundles incubated in pancreatic tumor-conditioned media, and in mice inoculated with pancreatic tumor cells. All animal and human experimentation were done in compliance with institutional regulations, including IACUC approval (protocol 202011171 at UF), IRB approval (BMC-06-015, A08-M66-12B), and written informed consent.

Results: Inducing mPT in single mouse muscle fibers using 300 nM Bz423 increased mROS and this was prevented by either an mPT inhibitor (1 mM TR002) or 5 mM mitoTEMPO. Similarly, Bz423 increased Casp3 activity and this was prevented by co-treating with TR002 or Ac-ATS010-KE. Incubating single mouse muscle fibers with 300 nM Bz423 for 24 h reduced muscle fiber diameter by ~20% and this was prevented by inhibiting mPT (TR002), inhibiting mROS, or inhibiting Casp3 (Fig 1). Treatment of isolated mouse skeletal muscle mitochondria with 25-50 mM Bz423 caused a complex I-specific respiratory impairment (Fig 2). Bz423 treatment of single mouse muscle fibers also reduced AChR content and fragmented the AChR cluster at the muscle endplate and this was prevented by inhibiting mPT or Casp3 (Fig 3). We found that the time to mPT in the CRC assay was reduced in muscle bundles from the vastus lateralis of elderly men and women (Fig 4). CRC was also reduced following an acute 60 min incubation of mouse muscle bundles in pancreatic tumor-conditioned media and in mice inoculated with pancreatic tumor cells (Fig 5).

Conclusions: Collectively, our results show that inducing mPT in skeletal muscle causes muscle atrophy, AChR cluster dismantling, and a complex I-specific respiratory impairment, which are common muscle phenotypes seen with aging and a variety of disease conditions. Consistent with mPT being relevant to muscle pathology with aging and disease, we also show that mitochondria are sensitized to mPT in aging and in response to tumor-host factors in cancer cachexia.

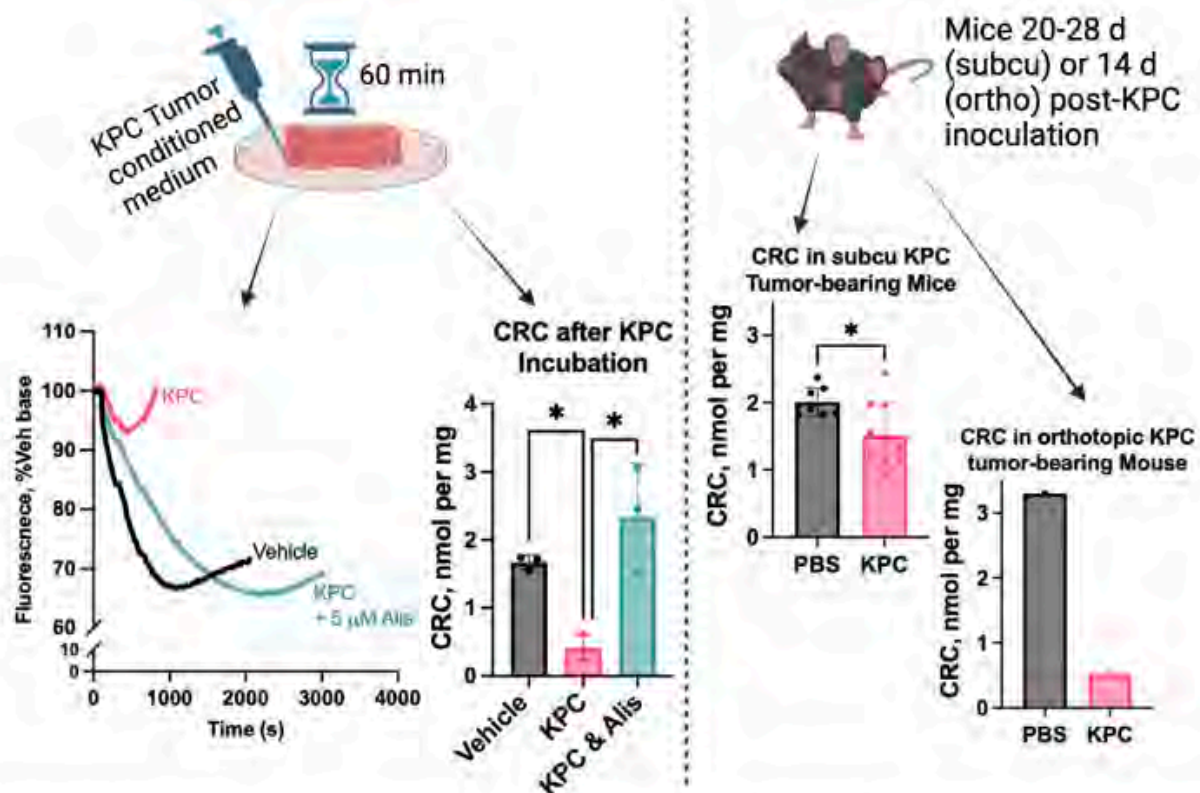


Figure 5. Tumor-host factors reduce the Ca^{2+} threshold for mPT. We incubated tibialis anterior myosin-depleted (ghost) muscle fiber bundles in KPC pancreatic tumor conditioned medium (50% diluted in DMEM) or Vehicle for 60 min at 4C. We then ran the muscle bundles through a Calcium Retention Capacity (CRC) assay. The KPC conditioned medium reduced CRC versus Vehicle and this was prevented by an mPT-inhibitor (5 μ M of the CypD-inhibitor Alisporivir [Alis]) (n=3 mice per treatment) (ANOVA). Similarly, mice bearing KPC tumors (n=10 KPC male mice for subcutaneous model; n=1 KPC female mouse for orthotopic model) had a reduced CRC versus PBS injected mice (n=8 male mice PBS for subcu, n=1 female mouse for orthotopic PBS) (compared by t-test). *P<0.05.

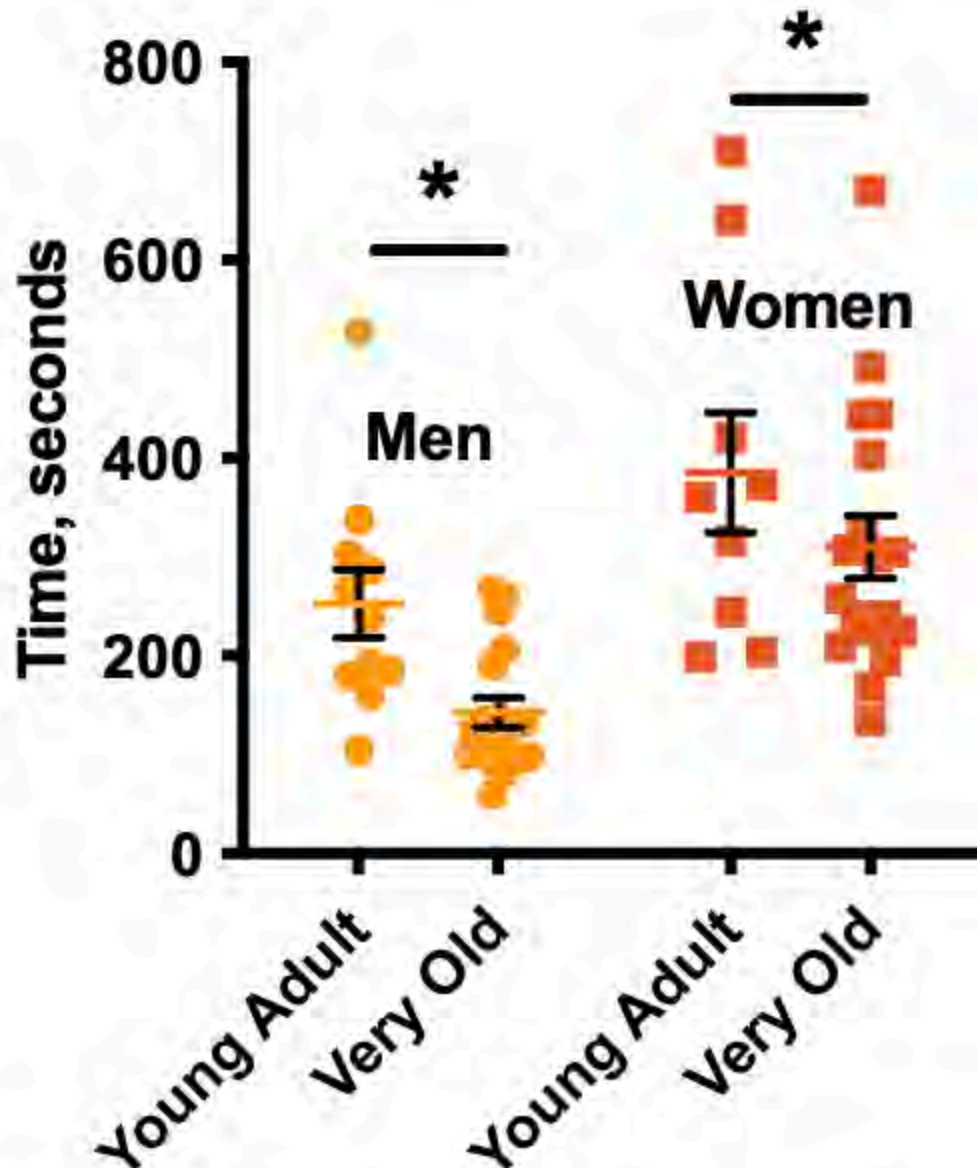


Figure 4. Bergstrom needle biopsies from the vastus lateralis muscle were obtained from young adult (20-30 y) and very old (76-89 y old) men and women. Saponin-permeabilized muscle bundles that had been depleted of myosin (so-called ghost bundles) were used in a Calcium Retention Capacity (CRC) assay using Ca^{2+} Green 5N. There was a main effect with no sex interaction for a longer time to reversal of the Ca^{2+} trace on the CRC assay in very old men and women versus their young adult counterparts. * $P < 0.01$ vs Day 0 (two-way ANOVA).

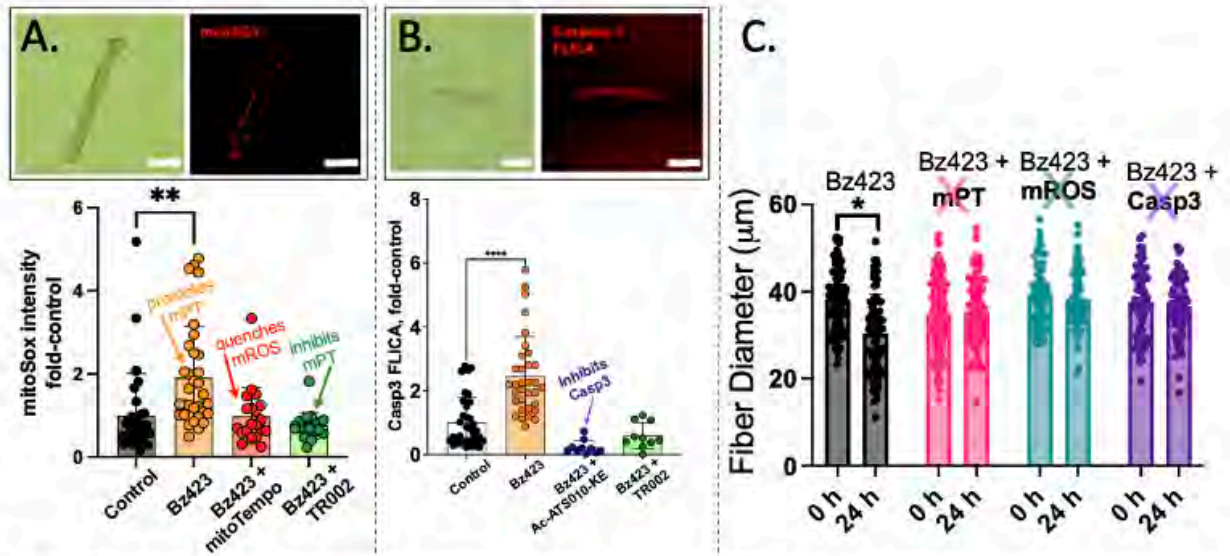


Fig 1. To determine if mPT causes atrophy in skeletal muscle fibers and its dependence upon mROS and Casp3, single mouse FDB muscle fibers were treated for 24h with 300 nM Bz423 (mPT-inducer), 300 nM Bz423 + 1 μM TR002 (mPT-inhibitor), 300 nM Bz423 + mitoTEMPO (mROS scavenger) or 300 nM Bz423 + 20 μM Ac-ATS010-KE (Casp3 inhibitor). mPT increased (A) mROS & (B) Casp3 activity, and induced atrophy that required both mROS and Casp3 activation (C). Biological replicates = 2-4. *** $P < 0.001$ and **** $P < 0.0001$ versus Day 0 (nested one-way ANOVA).

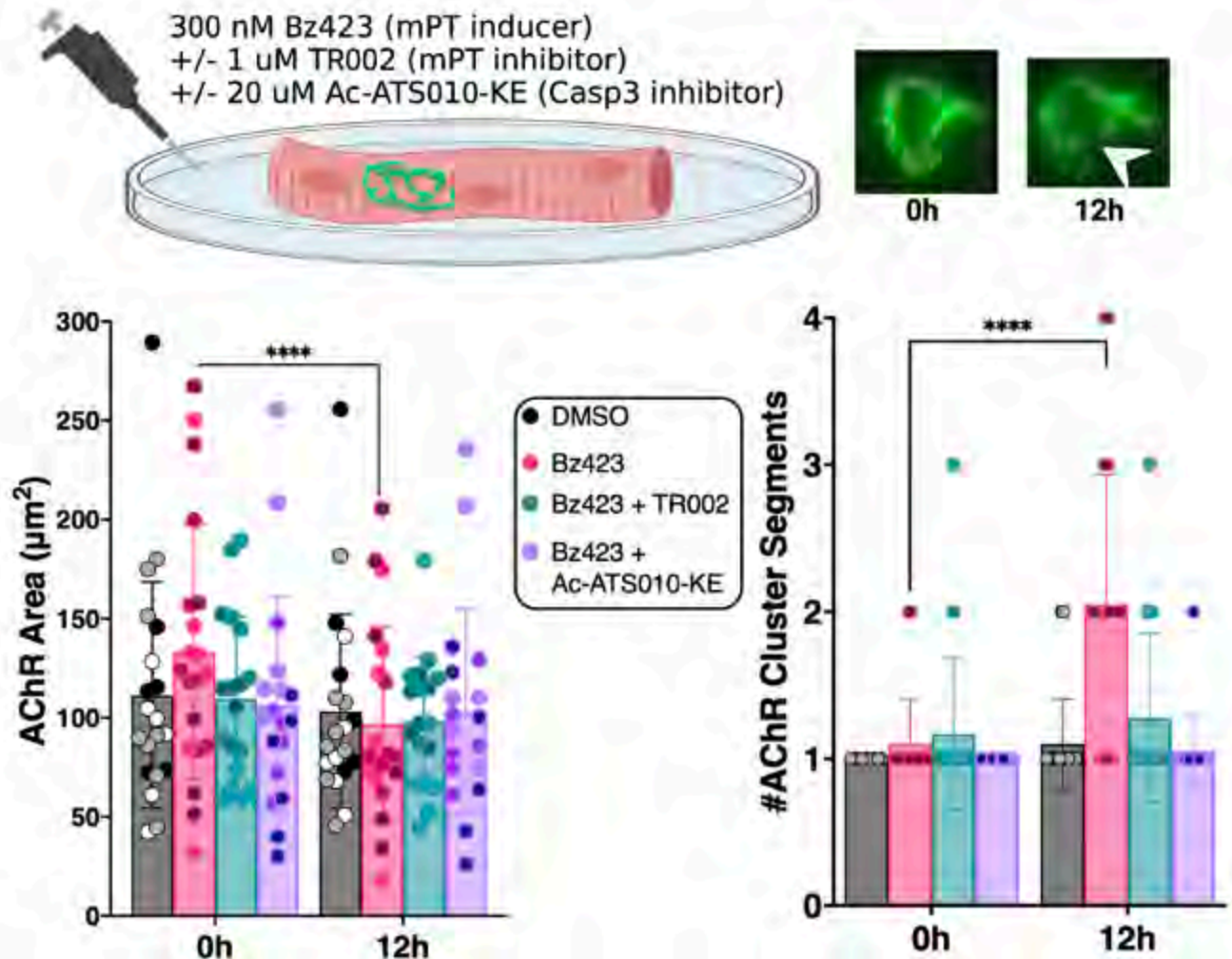


Figure 3. mPT causes AChR dismantling at the muscle endplate. AChRs on flexor digitorum brevis muscle fibers were labeled with AF488-bungarotoxin, treated with 300 nM Bz423 (mPT-inducer) +/- 1 μ M TR002 (mPT inhibitor), and imaged for 12 h. Images in upper right show a single endplate at time 0 and 12 h following treatment with Bz423. The white arrowhead shows an area of the AChR cluster that has fragmented and has reduced AChR intensity vs time 0. Graphs based on n=3 biological replicates (5 fibers/replicate) show that mPT reduced AChR area and induced AChR cluster fragmentation. **** P <0.0001 vs Day 0 (nested one-way ANOVA).

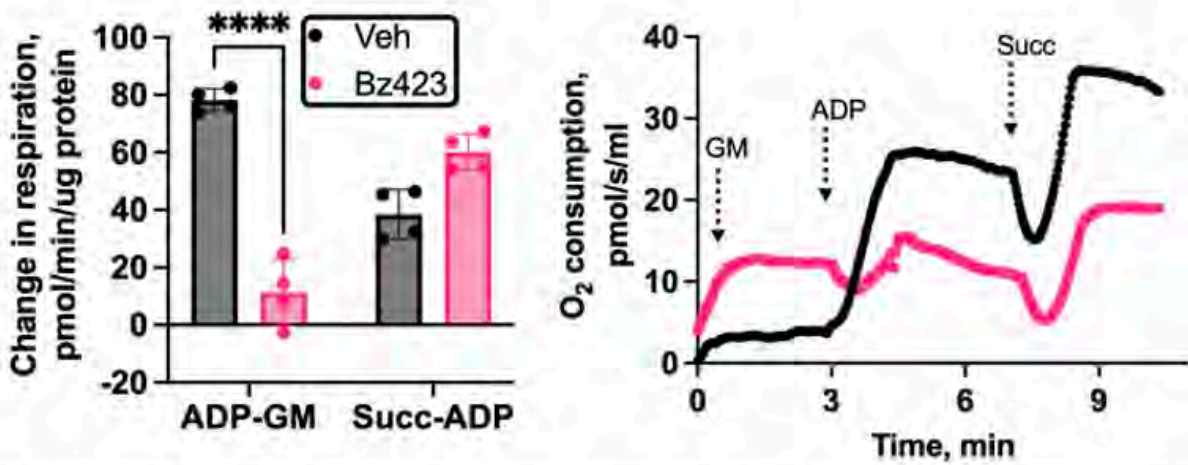


Fig 2. Treatment of skeletal muscle mitochondria with 50 μ M of the mPT-inducer Bz423 causes a complex I-specific respiratory defect. GM = 10 mM glutamate, 2.5 mM malate; +ADP = 2 mM adenosine diphosphate; +10 mM Succ = succinate. **** $P < 0.0001$ versus Vehicle (one-way ANOVA).

SA26

Using the gut brain axis to improve obesity treatment

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Obesity is now being recognised as a neurological disease that is associated with serious morbidity and increased mortality. Understanding gastro intestinal signalling to the subcortical areas of the brain offer a view on a therapeutic window for obesity which promises better clinical benefits as well as lower side effects.

Bariatric surgery is a good model to investigate gut brain signalling in humans and rodents, because it provides major changes in appetite with subsequent weight loss maintenance. Following gastric bypass, pleiotrophic responses from the gastro intestinal tract may contribute to improved appetite reduction, long-term lowering of body weight, glycaemic control and improvements in end organ damage.

The new third-generation medications based on optimising gut brain signalling are however now facilitating a revolution. These medications appear to address many of the diseases leading to obesity at their origins. Treating obesity as a chronic disease with effective therapies allows the disease to come under control and remain under control as long as the therapies continue. The impact of effectively treating obesity will reduce the symptoms of obesity such as excessive appetitive behaviour, but it will also reduce the complications of obesity which will have far-reaching benefits for the individual, healthcare systems, and wider society.

SA27

TGR5-dependent host-microbiome interactions reestablish epithelial intestinal homeostasis following colon injury.

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Introduction: Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic relapsing disorder characterized by inflammation of the gastrointestinal tract. Ulceration of the intestinal epithelium is a central factor in IBD, and preservation of its homeostasis appears crucial to prevent disease progression. A well-established feature of IBD is gut microbiome dysbiosis, which is characterized by a shift in microbial composition, leading to alterations in bacterial metabolites. Among these metabolites are secondary bile acids (BAs), pivotal in regulating intestinal and host physiology. BAs, initially synthesized in the liver as primary BAs and released into the intestine postprandially for lipid solubilization, undergo reabsorption and recycling in the small intestine. However, a fraction reaches the colon, where resident bacteria catalyze primary BA conversion into a variety of secondary BAs with different biological activities. Of particular importance are BA 7 α -dehydroxylating bacteria which convert the primary BAs cholic acid and chenodeoxycholic acid into deoxycholic acid and lithocholic acid, respectively, which are potent agonists of the BA-responsive membrane receptor Takeda G-coupled receptor 5 (TGR5). TGR5 activation drives multiple host processes, including gut hormone secretion, immunomodulation and stem cell-induced intestinal renewal. Since secondary BA production is diminished in IBD patients, we hypothesized that restoring 7 α -dehydroxylated BA levels may enhance the regenerative capacity of the colonic epithelium in mouse models and human patients with impaired or delayed intestinal repair capacity.

Aims and objectives: In this study, we investigated whether restoration of 7 α -dehydroxylated BA levels through colonization with the most characterized human-derived 7 α -dehydroxylating bacterium, *Clostridium scindens* (*C. scindens*), could re-establish epithelial intestinal homeostasis following colon injury. In addition, we analyzed omics datasets from UC patients and non-IBD individuals to translate our results to humans.

Methods: We colonized gnotobiotic Oligo-MM¹² and conventional C57BL/6J specific-pathogen free (SPF) mice with *C. scindens* and quantified fecal BAs. In both mouse models, colonic lesions were induced by administration of dextran sulfate sodium (DSS) and disease severity was assessed. SPF TGR5 knock-out mice and wild-type controls were also subjected to chemically-induced experimental colitis to evaluate the impact of the BA receptor TGR5. In addition, we reanalyzed public multi-omics datasets from a cohort of UC patients and controls to increase the relevance of our preclinical results.

Results and conclusion: Our study revealed that the amendment of *C. scindens* confers protection to DSS-induced colitis in both a prophylactic and therapeutic manner. Mechanistically, *C. scindens* improved the integrity of intestinal epithelium by promoting TGR5-mediated tissue regeneration. Finally, we observed that UC patients displayed dampened intestinal cell renewal and differentiation, and genes involved in those pathways showed a robust positive correlation with 7 α -dehydroxylated BAs levels. Our study reports *C. scindens* administration as a promising biotherapeutic strategy to foster epithelial regeneration and healing following colon injury by restoring the secondary to primary BA ratio.

SA28

Exploring fructose kinetics in Type 2 Diabetes: preliminary data from the ERIE trial

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Background and aims

Dietary fructose consumption has increased substantially in recent decades and is associated with higher incidence of obesity, type 2 diabetes (T2D) and other non-communicable diseases. However, causality and underlying mechanisms remain to be elucidated. Therefore, the 'Effect of dietary fructose on fructose kinetics in type 2 diabetes' (ERIE) trial is being performed. The trial aims to investigate fructose metabolism, including microbial fermentation, and its effects in T2D individuals, through examining oral fructose handling in T2D subjects receiving a high- or low-fructose diet.

Materials and methods

The ERIE trial, a double-blinded, isocaloric randomized controlled trial, aims to recruit 40 non-insulin dependent T2D participants on stable metformin therapy. Participants are randomly assigned to group A or B, receiving either a high- (100g/day) or a low-fructose (30g/day) diet for 4 weeks. Primary endpoints include changes in oral fructose handling, assessed through a fructose challenge test (FCT) by ingesting 1g/kg bodyweight unlabeled fructose and 120mg ¹³C₆-labeled fructose, in relation to metabolic markers such as HOMA-IR. Preliminary data from up to 18 participants were analyzed using multiple non-parametric tests, comparing deltas (Δ) of pre- and post-intervention results between both groups.

Results

In groups A and B, 45.5% and 28.6% of participants were female, respectively. Median age [IQR] was 65 years [56-68] in group A and 60 years [59-66] in group B. Median BMI [IQR] was 29.3 kg/m² [27.1-33.8] in group A and 29.9 kg/m² [27.9-32.2] in group B. Group B exhibited a significantly faster peripheral appearance of ¹³C₆-fructose and reached higher concentrations after 30 minutes with a median Δ [IQR] of 0.93 μ M [0.89-1.45], compared to 0.02 μ M [-0.46-0.45] in group A ($p=0.012$). Concurrently, group B showed a trend towards increased peripheral appearance of unlabeled fructose with a median Δ [IQR] of 207.70 μ M [144.26-271.14] after 30 minutes, compared to 1.14 μ M [-25.19-72.78] in group A ($p=0.178$). Uric acid levels in group B exhibited a significant rise throughout the FCT, with a median Δ [IQR] of 49.0 μ M/l [41.5-69.5] after 150 minutes, compared to -8.0 μ M/l [-14.0-23.0] in group A ($p<0.001$). No changes in glucose levels were observed in either group, with current inclusion numbers.

Conclusion

The accelerated and heightened peripheral appearance of fructose in group B, compared to group A, following ingestion of an equivalent oral fructose dose pre- and post-intervention, suggests altered fructose absorption in group B. This aligns with a significant increase in uric acid levels in group B, indicating enhanced hepatic fructose metabolism. These results show that varying levels

of fructose consumption affect acute fructose metabolism. Prolonged exposure to high fructose levels in the intestine may induce an upregulation of intestinal GLUT-5, facilitating increased fructose absorption. This can have negative metabolic effects by elevating fructose levels reaching the liver, thereby augmenting hepatic fructose metabolism. This can lead to increased uric acid production and fatty acid synthesis. Despite the trial's blinding, preliminary data suggest that group B adhered to the high fructose diet. These results provide novel insights into fructose metabolism in individuals with T2D, highlighting potential deleterious metabolic consequences likely associated with increased fructose consumption.

SA29

The gut-brain GLP-1 axis: role in limiting nutrient uptake and countering obesity

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The gut endocrine system comprises a collection of enteroendocrine cells scattered throughout the intestinal epithelium, producing hormones that signal locally within the gut and distantly at tissues such as the brain and pancreas. In the field of diabetes and obesity, therapies based on GLP-1 are now in widespread clinical use, and dual/triple agonists such as GLP1/GIP and GLP1/GIP/glucagon are showing even greater efficacy.

The study of GLP-1 secretion can now be performed using human intestinal organoids, engineered by CRISPR-Cas9 to label specific enteroendocrine cell populations or to knock out genes of interest. Application of live cell imaging, electrophysiology and transcriptomics to these organoid model has identified signalling pathways of particular interest including those activated by glucose, bile acids and free fatty acids. Whereas glucose stimulates enteroendocrine cells as a consequence of its electrogenic uptake via SGLT1, bile acids and free fatty acids target specific G-protein coupled receptors on the cell membrane, most notably GPBAR1 and FFAR1.

Understanding signalling in the gut brain axis provides a foundation for identifying new drugs for type 2 diabetes and obesity that act by targeting gut endocrine cells, thus mimicking the gut endocrine consequences of bariatric surgery.

SA30

Time-Of-Day Dependent Metabolic Response to Exercise

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Exercise is a potent energetic stressor and a major counter measure against metabolic disease. Timing of energetic stressors over the day may specify close alignment between tissue clocks and promote coherent and efficient temporal gating of metabolic processes. My team have integrated emerging omics technologies to interrogate biorhythms and physiological responses to energetic stressors. This led to an atlas of exercise metabolism, in which we mapped global metabolite responses of multiple tissues after exercise. Thus, we have made the surprising discovery that the therapeutic response to exercise is time-dependent. In fact, we have also shown that timing of exercise or meals throughout the day affects metabolism in people with obesity or type 2 diabetes, but the mechanism(s) are unknown. My team is advancing the notion of “chrono-exercise”. We integrate omics approaches with physiological phenotyping and downstream mechanistic analyses, to optimize metabolism by timing energetic stressors to the peak times of metabolic rhythms to positively impact cardiometabolic health. In lecture, I will present emerging evidence that timing may optimize the adaptive response to exercise and improve health outcomes. Chrono-exercise may optimize metabolism by timing energetic stress during the day to improve insulin sensitivity and glycemia, and thereby positively impact metabolic health.

SA31

Optimising concomitant metformin and exercise treatment by precision timing to improve glycaemic regulation in people with Type 2 Diabetes

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Exercise is beneficial for several organ systems in the body and increases insulin sensitivity [1]. However, previous evidence suggests that the timing of exercise in people with Type 2 Diabetes may have opposing outcomes on glycaemia [2]. This may be particularly relevant in people also being prescribed metformin [3], the most prescribed anti-hyperglycaemic medication for people with Type 2 Diabetes and often recommended concomitantly along exercise. Although exercise improves glycaemia, this effect is inhibited when undertaken alongside metformin ingestion [4,5]. Metformin intake also appears further inhibit the beneficial response to exercise, including ablating improvements in insulin sensitivity and skeletal muscle mitochondrial protein synthesis and respiration [6,7]. Therefore, we hypothesise that the skeletal muscle signalling response to exercise is disrupted by metformin and that it may be possible to optimise recommendations for when to take metformin together with exercise. To test this, we performed a remote crossover study, 9 male and 9 females with T2D undergoing metformin monotherapy completed 2-week baseline, six weeks randomly assigned to morning (7-10am) or evening (4-7pm) exercise (30 mins), with a two-week wash-out period. Acute AUC glucose was significantly lower ($p=0.01$) in participants taking metformin before breakfast ($152.5\pm29.95\text{mmol/L}$) compared with participants taking metformin after breakfast ($227.2\pm61.51\text{mmol/L}$) only during the morning exercise arm. Our data indicates that morning moderate exercise lower glucose levels in people with T2D on metformin, especially when metformin is taken before breakfast. We also analysed skeletal muscle biopsies from seven healthy lean men that completed one bout of single-leg exercise with a contralateral control-leg after acute metformin/placebo supplementation [8]. After fasting overnight, participants had biopsies taken from the vastus lateralis in both legs and then received either 1.5g of metformin or a placebo with breakfast. They then rested for 4½ hours and had biopsies taken from both legs. Then, they did a 40-minute knee extensor exercise at 80%PWL followed by another biopsy. RNA sequencing analysis revealed that metformin supplementation inhibited the transcriptomic response to exercise by reducing the total number of differentially expressed genes in response to exercise. After exercise, 53 genes were upregulated in the placebo trial, while only 17 genes were upregulated in the Metformin trial. Importantly, the transcription factor NR4A3 was upregulated in response to exercise during the placebo trial (adj. $p=0.011$), but not when participants consumed metformin (adj. $p=0.234$). In Human skeletal muscle myotubes (HSM), metformin (10µM) did not change NR4A3 expression (RT-qPCR, 1.2-fold-change, $p=0.536$) compared to the control. As expected, exercise mimetic Ionomycin (8µM) significantly increased NR4A3 expression (4.3-fold-change, $p<0.001$), however concomitant metformin incubation significantly reduced the NR4A3 response (2.4-fold-change, $p=0.002$). In summary, our preliminary

data suggest that taking metformin before breakfast when combined with morning moderate intensity exercise lowers blood sugar compared to other treatment timings. Our mechanistic investigations reveal that *NR4A3* response to exercise in skeletal muscle is inhibited by metformin, which is likely underlying the apparent inhibition of exercise-induced skeletal muscle glucose uptake with concomitant metformin. Our data suggest that it may be possible to optimised exercise timing alongside concomitant metformin to augment skeletal muscle glucose uptake.

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SA32

The complexities of characterising shift work, diet and blood glucose variability in a real-world setting

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undefined

The night-time economy contributes £93.7bn annually to the UK economy(1) and relies on extensive employment of night workers. The association between night work and adverse cardiometabolic health is of increasing concern. Shift workers, compared to day workers, are more likely to be diagnosed with type 2 diabetes (T2D)(2) and shift workers living with T2D are reported to have higher glycated haemoglobin compared to day workers(3). Simulated shift work studies have significantly contributed to understanding how eating at night impacts physiology however, real world research is needed among shift workers. The Shift-Diabetes study (ISTCTN 11764942) is a mixed methods study with the aim to characterise current management of T2D in shift workers.

An observational study was conducted in 40 shift workers (working a combination of night and day shifts) living with T2D. Across 10-days, data was collected on blood glucose (continuous glucose monitor), diet (self-report diary), sleep and physical activity (actigraphy). The monitoring period covered night shifts, day shifts and rest days. Within person mean glucose concentration (MG), coefficient of variation (CV), mean absolute glucose (MAG), mean amplitude of glycaemic excursion (MAGE) and dietary intake (food choices, nutrient intake) were compared between 'behavioural day' types. A 'behavioural day' was defined as the period between two main sleep periods (sleep off set to sleep off set)(4) Figure 1. Behavioural day types were categorised as: night shift, day shift, rest after night shift and day off. A parallel qualitative study was conducted in 15 shift workers using semi-structured interviews to explore barriers and enablers to dietary behaviour during shift work(5).

The study sample was predominantly female (89.2%) and employed as a nurse or midwife (62.2%). The behavioural day duration was significantly longer when a night shift was worked (26.9 SD2.5hrs) compared to a day off (23.5 SD1.0hrs, $p < 0.001$). A rest after night shift day was shorter than all other day types (17.2 SD2.7hrs, $p < 0.001$). Energy intake (%) from confectionary was higher on a night shift compared to a rest after night shift (13.4 SD12.0% vs. 7.8 SD11.8%, $p = 0.013$). Caffeine intake was significantly different across all day types, except between day off and day shifts, with intake highest on night shifts (164.8 SD175.5mg/day). There were differences in the number of eating occasions, with night shifts having the highest occasions (7.0 SD2.2) and rest after night the lowest (3.4 SD1.6). No differences were observed for MG, MAGE or CV. MAG was higher for night shift compared to rest after night shift (3.4 SD1.0 vs. 2.9 SD1.6, $p = 0.029$). The qualitative study found shift workers wanted to make healthier food choices during night work but were inhibited due to limited access to food and a lack of confidence in adjusting diet intake to shift schedules(5).

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The duration of behavioural day when dictated by type of shift can vary significantly and may impact dietary intake and blood glucose. Further research to understand how changes in behavioural day duration impact shift worker behaviours and health are needed.

King's College London BDM Research Ethics Subcommittee (HR-19/20-14630)

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SA33

Examining the Contribution of NR1D1 to Adaptive Metabolic Response

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The circadian system is essential to normal regulation of energy balance and metabolic health. Local circadian mechanisms shape rhythmic transcriptional and translational events in the brain and key peripheral tissues to cope with the daily switch between fed and fasted states. Importantly, clock function and individual components of the circadian clock contribute to homeostatic mechanisms which dictate physiological response to acute and chronic shifts in energy state and can mitigate response to metabolic perturbation, such as mistimed food intake. Here I will discuss some of our work examining the role of Rev-erba (Nr1d1) in adaptive metabolic response to both acute energy deficit and chronic surplus in mice.

SA34

Reversing pericyte-mediated capillary constriction: a therapeutic target for restoring cerebral blood flow in dementia-causing conditions.

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Although the generation of amyloid beta oligomers and plaques, and of hyperphosphorylated tau, are hallmarks of Alzheimer's disease (AD), it has been shown that an earlier change in AD is a decrease of cerebral blood flow (CBF) by ~50% in affected brain areas. Cerebral blood flow can also decrease in vascular dementia.

By imaging capillary diameter in neuropathological biopsy sections from the right prefrontal cortex of humans developing AD we previously showed that capillaries in humans with AD are constricted as a result of pericyte contraction (Nortley et al., 2019). A similar pericyte-mediated constriction of capillaries in vivo was seen using 2-photon imaging through a cranial window in anaesthetised AD mice (APP^{NL-G-F} mice) expressing dsRed in pericytes controlled by the NG2 promoter. In the mice we also showed that there was no constriction of arterioles or venules, and that the capillary constriction leads to a stalling of neutrophils near pericyte locations and to tissue hypoxia.

In rodent brain slices we found that applying exogenous oligomerised amyloid beta (EC₅₀~6 nM) led to a pericyte-mediated constriction of capillaries, that was generated by endothelin-1 (ET) release and activation of ET_A receptors (Nortley et al., 2019). This reflected reactive oxygen species generation, since it was reduced by NOX4 and NOX2 blockers (Nortley et al., 2019). ET-evoked pericyte contraction is amplified by a rise of [Ca²⁺]_i activating TMEM16A chloride channels, which generate a depolarisation that activates voltage-gated Ca²⁺ channels (Korte et al., 2022).

To attempt to use a re-purposed drug to reverse the capillary constriction in AD, we employed the voltage-gated calcium channel blocker nimodipine, which is licensed for human use in sub-arachnoid haemorrhage. Giving nimodipine, either intravenously, or in the drinking water from 1.5 to 4 months of age, led to a relaxation of pericytes and arteriolar smooth muscle cells, an increase of cerebral blood flow, a block of the stalling of neutrophils in capillaries, and a partial reversal of the tissue hypoxia.

Other possible therapeutic targets for reversing or preventing a decrease of cerebral blood flow in AD or in some types of vascular dementia would include the mechanisms leading to endothelin

release in AD, ET_A receptors, TMEM16A channels, and the concentration of cyclic nucleotides in contractile mural cells where a rise of cGMP and cAMP concentration (for example in response to a guanylate cyclase receptor agonist: Nortley et al., 2019) leads to vasorelaxation. Agents that increase cerebral blood flow are likely to be useful as adjunct therapies with other dementia-targeting drugs.

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SA35

Lasting, low amplitude responses characterize the remodeled neurovascular coupling and Ca²⁺ signaling in the aged awake mouse brain

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Brain aging is associated with reduced vascular reactivity, exacerbated brain energy deficits and cognitive decline. However, it is still unclear how normal aging impacts neurovascular function and its underlying mechanism, especially in the awake brains. It is also unknown whether arteries and capillaries undergo different age-related structural and functional remodeling. Using laser speckle contrast imaging and two-photon imaging in awake, behaving young adult and aged mice, we show that neurovascular coupling (NVC) responses in the aged brain are prolonged, with reduced amplitudes, and this is more pronounced at the capillaries than at the arteries. Compared with anesthetized state, the total flow change of NVC at both arteries and capillaries are preserved in the awake aged brains. Furthermore, the NVC response is mediated by Ca²⁺ signaling in vascular mural cells, i.e. vascular smooth muscle cells and pericytes. We revealed a different Ca²⁺ kinetics of vascular mural cells in young adult and aged mice. The rate of calcium transport and the calcium sensitivity of vascular mural cells are reduced in aged mice, which explains the reduced and prolonged vasodilation. Structurally, we further revealed the retraction of transitional point from contractile to non-contractile mural cells at aged capillaries. Lastly, the vascular and Ca²⁺ responses triggered by spontaneous behavior such as locomotion also exhibits consistent prolonged characteristics. In all, our data suggests that the altered NVC responses in the aged awake brain is associated with remodeling of Ca²⁺ signals and vascular coverage of contractile mural cells. Novel interventions to delay age-related brain pathology could be based on fully harnessing the power of the vascular remodeling mechanisms with age.

SA36

Capturing rapid changes in cerebral blood flow.

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Adequate neurovascular dynamics, hemodynamic resistance, and microvascular stiffness are key to maintaining healthy brain function, whereas associated abnormalities play a crucial role in developing neurodegenerative or cardiovascular diseases. Characterising these features is pivotal for research and diagnostics and can generally be achieved by measuring perfusion and diameter changes associated with indigenous, e.g. cardiac or neurovascular activity, or exogenous factors, e.g. pharmacological stimulation. Such changes, however, often propagate rapidly over short microvascular segments and, therefore, require high-speed, high-resolution imaging to characterise them in detail. To address this challenge, our group has developed High-Speed Laser Speckle Contrast Imaging [1,2] (HS-LSCI) – a dynamic light scattering imaging technique that allows full-field microvascular perfusion imaging at >5000 frames per second. Here, we will explain the theoretical and technical foundations of high-speed blood flow imaging, discuss potential applications and show some of the first results acquired from the mouse cortex. Specifically, we will present the analysis of cardiac-cycle associated perfusion changes in ageing mice and approach the topic of the spatiotemporal dynamics of neurovascular response caused by air-puff stimulation of the mouse whiskers. All experimental protocols in the relevant studies were approved by the Danish National Animals Experiments Inspectorate and conducted according to their guidelines and guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

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SA37

Neurovascular Impulse Response Function, Neuromodulation and Behavior

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Ascending neuromodulatory projections from deep brain nuclei generate internal brain states that differentially engage specific neuronal cell types. Because neurovascular coupling is cell-type specific and neuromodulatory transmitters have vasoactive properties, we hypothesized that the impulse response function (IRF) linking spontaneous neuronal activity with hemodynamics would depend on brain state.

To test this hypothesis, we used optical imaging to measure (1) release of neuromodulatory transmitters norepinephrine (NE) or acetylcholine (ACh), (2) Ca^{2+} activity of local cortical neurons, and (3) changes in hemoglobin concentration and oxygenation across the dorsal surface of cerebral cortex during spontaneous neuronal activity in awake mice.

Fluctuations in total hemoglobin (HbT), reflective of dilation dynamics, were well predicted by a weighted sum of positive Ca^{2+} and negative NE contributions, while ACh signals were largely redundant with Ca^{2+} . IRF varied in time and depended on the arousal (dilation of the eye pupil, whisking) captured by NE but not ACh. During high arousal, the dynamic nature of IRF resulted in the loss of hemodynamic coherence between cortical regions (known as “functional connectivity” in BOLD fMRI studies) despite coherent behavior of the underlying neuronal Ca^{2+} activity.

We conclude that neurovascular coupling is a dynamic phenomenon that reflects NE neuromodulation and behavior. Dynamics of IRF challenges the metric of functional connectivity because the loss of hemodynamic coherence can be falsely interpreted as neuronal desynchronizations.

SA38

Direct activation of renal, Ghrelin-family GPR39 receptors reduces urinary concentration capacity

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Fasting results in significant changes in the distribution of body fluids, leading to a decrease in blood pressure and circulatory volume. Lately, several primary gastrointestinal hormones and orphan receptors in taste and olfaction have been implicated in the regulation of renal function¹⁻⁵, and hence, one can speculate that appetite-regulating signalling might be responsible for the circulatory volume contraction observed in response to anorectic states. Activation of the orphan receptor GPR39 of the ghrelin receptor family in the gastrointestinal tract results in local GLP-1 release and a following decrease in food intake and weight reduction in mice⁶. In the current study, oral gavage of a selective GPR39 agonist (Cpd1324) increases the water intake in C57BL/6J mice. The effect is dose-dependent fashion, and results in over two-fold increase in 24-hour water intake relative to vehicle controls, an effect that was completely absent in global GPR39 deficient mice. Cpd1324 markedly reduced the urinary concentrate capacity in C57BL/6J mice after eight hours of water restriction (1927.0 ± 129.3 mosmol/kg, $n=5$) compared to vehicle controls (3196.0 ± 139.9 mosmol/kg, $n=5$, $p<0.001$). In *ex vivo* perfused cortical collecting ducts, GPR39 (10 μ M) directly counteracted the AVP-induced water permeability, corresponding to a Cpd1324-induced around 50% reduction in phosphorylated AQP2 (S256) abundance assessed by immunoblotting of renal tubule suspension. GPR39 activation also reduced the amount of phosphorylated NCC in distal convoluted tubules, with a parallel increase in urinary K⁺ excretion. These data suggest that the Cpd1324-induced drinking behaviour is secondary to a urinary concentration deficiency caused by opposing AVP-induced water reabsorption in the collecting duct. Moreover, we propose that a GPR39-dependent urine concentration defect and reduced NaCl reabsorption in the distal convoluted tubule might explain the circulatory volume contraction observed during fasting.

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SA39

Transcriptomics and Proteomics Data from Micro-dissected Renal Tubules Identifies the Distributions of All GPCRs Along the Renal Tubule

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A broad objective in systems biology is to identify every gene expressed in every cell type in the body. In kidney, we have used RNA-seq and protein mass spectrometry in microdissected kidney tubule segments to map expression of all genes in all renal tubular epithelia. Because collecting ducts (CDs) contain a mixture of principal cells (PCs) and intercalated cells (ICs), we have resolved separate transcriptomes in these cell types using single-cell RNA-seq. Data have been provided to the kidney research community through web-based sharing of curated data sets (<https://esbl.nhlbi.nih.gov/Databases/KSBP2/>). These data sets can be browsed, searched or downloaded, allowing them to be ‘mined’ to tie gene expression patterns to physiological functions of different renal epithelial cells.

Because signaling through GPCRs is vital to control of transport and metabolism in the kidney, we have used the data to curate all GPCRs present in mammalian genomes amounting to 790 genes (<https://esbl.nhlbi.nih.gov/Databases/GPCRs/>) and mapped them to each of 14 renal tubule segments and to PCs and ICs of the CD (<https://esbl.nhlbi.nih.gov/Databases/GPCRs/TubuleTPM.html>). One area of interest is regulation of aquaporin-2 (AQP2) in principal cells. Although it is well known that regulation of AQP2 trafficking and transcription are dependent on increased cAMP secondary to the G_s-coupled vasopressin V2 receptor (Avpr2), two other G_s-coupled receptors were identified in PCs. These are the prostaglandin EP4 receptor (Ptger4) and the calcitonin-related receptor-like receptor (Calcrl), which binds adrenomedullin in collecting duct PCs. Ligand interactions with both receptors have been shown to increase cAMP and water permeability in collecting ducts, pointing to alternative treatment modalities in X-linked nephrogenic diabetes insipidus due to mutations in Avpr2. In addition, some G_s-coupled receptors expressed in collecting duct are intercalated-cell-specific (namely, the secretin receptor [Sctr] and β adrenergic receptors [Adrb1 and Adrb2]), which have been shown to regulate acid-base transport. In addition to the vasopressin V2 receptor, another vasopressin receptor is also expressed in the collecting duct (viz. the V1a receptor), and is found only in intercalated cells.

Aside from the renal collecting duct, the epithelial cells of the nephron, including the subsegments of the proximal tubule, the thin and thick limbs of Henle’s loop, macula densa and distal convoluted tubule all have been found to express combinations of GPCRs that are indicative of intricate regulation of transport and metabolism (Poll et al. Am J Physiol 2021; 321: F50-F68), much of which remains to be investigated.

SA40

Secretin: a key regulator of urine production and acid/base excretion

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In 1902 Bayliss and Starling discovered the first peptide hormone, secretin. Secretin is released in response to food intake and the postprandial effects of secretin in the gastrointestinal system are well explained and understood. Here, its effects are well-understood: it activates pancreatic and bile duct bicarbonate secretion and inhibits gastric acid secretion. Beyond the gastrointestinal system, the secretin receptor is also expressed in the kidney, where it has been implicated in water conservation, although the mechanism remained uncertain.

We have discovered that secretin via its receptor controls renal base excretion by activation of the base secretion in beta-intercalated cells of the kidney collecting duct. Notably, secretin-release is augmented during acute metabolic alkalosis. Consequently, loss of the secretin receptor impairs the kidneys' ability to increase renal base excretion and compensate an acutely imposed metabolic alkalosis.

Additionally, we have uncovered that secretin also strongly modulates the glomerular filtration rate by selective vasodilation of the vas efferens, thereby controls urine production. Both effects - decreased renal acid excretion and decreased urine production - can be viewed as beneficial in the postprandial state by normalizing the postprandial blood alkalinity and redistribute more volume to the gastrointestinal system for digestive secretions.

Physiological quantification of GI hormones and their mechanisms of release

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The gastrointestinal tract is a central organ for regulation of appetite and metabolism being part of the gut-brain axis and due to its production of hormones regulating digestion, gastrointestinal motility and secretion, food intake and glucose homeostasis. The gut hormones affecting appetite and glucose metabolism are of particular interest due to their pharmacological potential. Thus, agents based on glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) have proven effective in the treatment of type 2 diabetes mellitus and obesity. However, side effects of GLP-1 analog treatment (nausea, vomiting, and diarrhea) affect some patients. These side effects might be avoided by targeting endogenous hormone secretion reflecting normal physiology more closely. Identifying mechanisms that can be engaged to increase endogenous gut-derived hormone secretion is thus an important area of research.

Gut hormones are secreted from enteroendocrine cells scattered throughout the gastrointestinal tract. Knowledge about how the enteroendocrine cells sense and react to the different nutritional components is essential but is still limited and we have only recently begun to understand how macronutrients and their digestive products stimulate the secretion. The nutrient sensing mechanisms range from electrogenic transporters, ion channels and nutrient-activated G-protein coupled receptors activating a variety of intracellular signaling pathways which all represent relevant targets.

Using the perfused intestinal rat model, it is possible to obtain detailed knowledge of these mechanisms by evaluating nutrient handling and absorption, the effect of blocking or stimulating specific transporters and receptors, and the resulting hormone secretion in different sections of the intestine. Using this highly physiological relevant model, we now have strong indications for a role for both Sodium-Glucose Transporter 1 (SGLT1) and the K⁺_{ATP} channel activity in carbohydrate-induced GLP-1 secretion. Lipid-induced GLP-1 secretion has been linked to the G-protein-coupled receptors; GPR40 and GPR119, and protein-induced GLP-1 secretion is believed to depend on Peptide Transporter 1 (Pept1) mediated absorption and activation of the Calcium-Sensing Receptor (CaSR). Expression analysis and stimulation experiments indicated that numerous additional mechanisms may be at play, so more work is clearly needed.

SA42

The effects of glucagon on the kidney distal convoluted tubule and blood pressure

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Introduction. The NaCl cotransporter (NCC) is expressed in the kidney distal convoluted tubule (DCT) and is a widely used pharmacological target for hypertension treatment. NCC is also important for plasma potassium (K⁺) homeostasis, as changes in NCC activity can influence the degree of electrogenic K⁺ secretion in the latter portions of the renal tubule. Changes in NCC phosphorylation track with NCC activity, with greater NCC phosphorylation correlating with higher NCC activity. Phosphorylation of NCC is increased in response to various stimuli, including changes in the extracellular K⁺ concentration, or after alterations in intracellular cAMP following vasopressin or adrenergic hormone stimulation of GPCRs. Database searching indicated that the GαS coupled glucagon receptor (GluR), which also signals via cAMP, was abundantly expressed in the DCT. We hypothesized that glucagon was a potent modulator of NCC activity, Na⁺ and K⁺ balance and ultimately blood pressure.

Methods. *Ex vivo* effects of glucagon on NCC phosphorylation status were assessed in mouse and human tissue. Involvement of selective signaling pathways downstream of the GluR were determined using pharmacological tools. *In vivo* effects of short-term or long-term glucagon administration on blood pressure, and Na⁺ and K⁺ balance was examined in mice.

Results. In mice, 30-min glucagon exposure increased NCC phosphorylation. In *ex vivo* kidney tubule suspensions glucagon increased NCC phosphorylation in a time and dose-dependent manner, an effect prevented by a GluR inhibitor. Glucagon increased NCC phosphorylation in human kidney slices. Selective pharmacological inhibition of adenylyl cyclase, protein kinase A, with no lysine kinases (WNK) and inward-rectifier potassium channels 4.1/5.1 or intracellular Ca²⁺ chelation antagonized the effects of glucagon on NCC phosphorylation. Glucagon effects were absent in tubules isolated from protein phosphatase 1 inhibitor-1 KO mice. In mice administered glucagon by osmotic mini-pump for 14 days, plasma glucagon was significantly higher and NCC phosphorylation 2-fold greater than the vehicle group. Plasma Na⁺ and K⁺ levels were not significantly different between groups. Despite the increase in NCC phosphorylation, preliminary studies suggest that glucagon prevents the ability of a high NaCl intake to increase blood pressure.

Conclusion. Glucagon is a potent stimulator of NCC phosphorylation and activity. The actions of glucagon in modulating electrolyte balance and blood pressure may be underappreciated and play an important role in human physiology/pathophysiology.

SA43

Aldo/MR therapeutic targeting, epigenetics and transcriptional regulation

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The mineralocorticoid receptor (MR) is a ligand-activated transcription factor from the nuclear receptor family. The physiological function of MR is the control of water and electrolyte balance, primarily via the regulation of sodium channels such as ENaC in epithelial cells of the kidney and colon. In addition, MR has strong inflammatory and pro-fibrotic properties and thus unfavorable impact on heart and kidney function. MR antagonists are widely established in the treatment of chronic heart failure and renal insufficiency. However, currently available compounds are associated with an increased risk of adverse effects such as hyperkalemia, which considerably limits their clinical applicability [1]. During the past two decades, mouse models with cell type-specific deletion of MR demonstrated that the pathological effect of MR is primarily mediated via cardiomyocytes, endothelial cells, and immune cells [2]. Selective blockade of the MR signaling pathway in these cell types could therefore separate the pathological inflammatory effects from the physiological function of MR.

Endothelial cells are considered a central hub of cell-cell communication in the heart. They form capillaries that are crucial to maintain the high oxygen and energy demand of cardiomyocytes. As endothelial cells represent the inner layer of the vasculature, they have an important barrier function, control the migration of immune cells from the bloodstream, and regulate tissue homeostasis [3]. Gene expression is controlled by transcription factors binding to enhancers and promoters. We created a comprehensive atlas of chromatin accessibility, histone modifications, and 3D chromatin organization and determined enhancer-promoter interactions in cardiac endothelial cells and confirmed the functional relevance of enhancer-promoter interactions by CRISPRi-mediated enhancer silencing. A systematic comparison of mouse models representing different cardiovascular risk factors identified MR as common regulator of pathological gene expression in cardiac endothelial cells and predicted direct MR target genes [4]. We conclude that epigenetic modulation may be a promising strategy towards selective targeting of MR-dependent pathological gene expression.

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SA44

Expression of functional mineralocorticoid receptor (MR) and G-protein coupled estrogen receptor (GPER) in human T lymphocytes

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Introduction

Aldosterone plays a key role in controlling blood pressure values by maintaining salt, water, and body fluid homeostasis. It exerts genomic effects that are mediated by activation of the mineralocorticoid receptor (MR) and 'rapid' or 'non genomic' effects that involve additional receptors as the G-protein coupled estrogen receptor (GPER). Excessive production of aldosterone generates an inflammatory state that is associated with cardiovascular and metabolic diseases that can be promoted by the involvement of innate and adaptive immunity. However, it is unknown if cells of the innate and adaptive immunity are endowed with aldosterone receptor(s) and are implicated in the inflammatory action of aldosterone.

Aims

Therefore, we sought for aldosterone receptors on human T lymphocytes and investigate whether aldosterone affects T cells activation.

Method

The expression of MR and GPER on human T cells was tested by measuring mRNA copy number by droplet digital PCR and immunoblotting from 7 healthy donors (HD). Peripheral blood mononuclear cells (PBMCs) from HD were exposed to different concentrations of aldosterone (from 10^{-10} M to 10^{-8} M) with or without MR antagonist (canrenone) and GPER antagonist (G36) under chronic and acute stimulation. We evaluated the effect of aldosterone CD8⁺ T cells by measuring IFN γ release with flow cytometry. We also sought for the expression of the cortisol inactivating enzyme 11 β -Hydroxysteroid Dehydrogenase Type 2 (11 β HSD2) in CD4⁺ and CD8⁺ lymphocytes.

Results

We detected the MR and GPER both at mRNA and protein level in CD8⁺ and CD4⁺ T cells. MR mRNA copy number (CD4⁺= 3061 \pm 4037 copies/50 ng of mRNA and CD8⁺= 3379 \pm 3112 copies/50 ng of mRNA) was at least 40-fold high that of the GPER (CD4⁺= 65 \pm 71.7 copies/50 ng of mRNA and CD8⁺=86.4 \pm 68.9 copies/50 ng of mRNA). At the protein level, the rank expression was GPER>MR in both CD4⁺ and CD8⁺ lymphocytes. Aldosterone significantly increased IFN γ release in CD8⁺ T-cell both under chronic and acute stimulation. Chronic exposure to aldosterone increased IFN γ production in a dose-dependent manner in CD8⁺ T cells by acting via the MR, as it was prevented by canrenone ($p < 0.0001$), while the rapid aldosterone action was slight reduced by G36 and

mimicked by G1. However, we found only a bare expression of mRNA for 11bHSD2 in CD4⁺ (4.2±8.3 copies/50ng mRNA) and CD8⁺ (3.6±6.3 copies/50ng mRNA) lymphocytes of HD subjects. Therefore, as the physiological concentration of cortisol in plasma is 10²-10³ fold higher than aldosterone, it could be that some effect elicited in vitro by aldosterone are in fact driven by cortisol.

Conclusions

In conclusion we found compelling evidence for the presence of MR and GPER receptors in human T lymphocytes and suggested that aldosterone and cortisol affect IFN γ release in human CD8⁺ lymphocytes.

SA45

Cardiometabolic consequences of adrenal gland dysfunction

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Overweight and obesity are major public health issues. Central obesity is associated with the risk of developing a metabolic syndrome. However, less than 20% of overweight people achieve sustainable weight loss, leading to repeated cycles of weight loss and regain, which are particularly associated with an increased risk of developing metabolic syndrome. Moreover, in postmenopausal women, estrogen deficiency promotes metabolic and endothelial dysfunction, further predisposing them to metabolic syndrome and its cardiovascular complications. Furthermore, leptin, an adipokine whose levels are higher in obese patients, especially in women, directly stimulates aldosterone production, suggesting that the adrenal glands could be a comorbidity factor in metabolic diseases. During estrogen deficiency, such as in menopause, there is an increase in Luteinizing Hormone (LH), due to the loss of negative feedback by sex steroid. In experimental studies, menopause increases aldosterone levels, an effect alleviated by estrogen treatment, while other studies have shown a decrease in aldosterone levels in postmenopausal women.

All these observations support a strong physiological and/or pathological link between the sex steroids' status (pre- or post-menopausal) and aldosterone synthesis and secretion. In turn, excess aldosterone could lead to cardiovascular and metabolic alterations

Our objective is to assess the role of the adrenal gland in postmenopausal cardiometabolic complications associated with cyclic weight variations.

For this purpose, female mice, both ovariectomized and non-ovariectomized, were subjected to three cycles of a high-fat diet/standard diet (yo-yo diet) or to a standard diet for 33 weeks. Functional and molecular explorations were performed at the end of each phase.

The yo-yo diet induced greater weight gain in ovariectomized mice compared to non-ovariectomized mice. Post-ovariectomy, the mice exhibited higher fasting blood glucose levels and circulating leptin levels, an effect that was more pronounced when the mice were subjected to the yo-yo diet. Ovariectomized mice on the yo-yo diet developed heart failure with preserved ejection fraction, which was prevented by the use of a mineralocorticoid receptor antagonist. Interestingly,

ovariectomized mice on the yo-yo diet showed an increase in adrenal gland weight, accompanied by significant morphological and functional remodeling of the adrenal cortex.

The absence of estrogens leads to cardiometabolic disorders that are worsened by a yo-yo diet but also to adrenal dysfunction, which could in turn contribute to the worsening of cardiometabolic functions.

SA46

Mineralocorticoid Receptors in Metabolic Syndrome

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The pathophysiology of cardio-metabolic diseases involves a delicate interplay between biological and environmental factors regulating energy homeostasis, water and electrolyte balance, as well as inflammation and oxidative stress in critical organs for cardio-metabolic health.

In this context, several studies clearly demonstrated a specific role of extra-renal mineralocorticoid receptors (MR) in controlling endothelial function, vascular tone, adipose tissue differentiation and inflammation, and heart physiology. In preclinical models, abnormal activation of MR in the vascular endothelial cells and in adipocyte favors the occurrence of several components of the metabolic syndrome, such as hypertension, obesity, and glucose intolerance. Moreover, it is well known that high circulating levels of aldosterone are associated with obesity and metabolic syndrome in humans, suggesting that altered activation of the MR in extra-renal tissues leads to important metabolic alterations. In this context, MR antagonists represent a promising approach to tackle cardiovascular and metabolic disorders occurring in the metabolic syndrome, even if there is still an important gap of knowledge about the metabolic effects of MR antagonists in clinical settings. This talk will discuss the complex interplay between the mineralocorticoid system, adipose tissue and endothelial cells and its role in the pathophysiology of cardio-metabolic diseases.

SA47

Hypoxia-inducible factors and brain metabolism: adaptation and pathology

Andrew Murray

undefined

Tissue hypoxia arises when there is a mismatch between oxygen delivery and tissue metabolic demand. As such, hypoxia can result from exposure to environmental hypoxia e.g. hypobaric hypoxia at high altitude, or under pathological circumstances when convective oxygen supply to the tissues is perturbed. In highly oxidative tissues, such as the brain, this can challenge energetic and redox homeostasis, but is associated with metabolic responses that might mitigate this challenge. The hypoxia-inducible factor (HIF) pathway acts as a major regulator of the tissue response to hypoxia, controlling the expression of thousands of genes, including many genes involved in metabolic function. The characteristic metabolic response of tissues includes the suppression of mitochondrial respiratory function and increased glycolytic flux. Components of the HIF pathway including *EPAS1* (encoding HIF2 α) have undergone natural selection in populations native to high-altitude regions including the Tibetan Plateau and the Andean Altiplano. The brain metabolic response to hypoxia, and the role of the HIF-pathway, can therefore be understood, in part, through studies of lowlanders and adapted highlander populations undergoing acute exposure to hypoxia. Conversely, emerging evidence suggests that metabolic disease might influence brain oxygenation and metabolic function. Hypersecretion of the pancreatic hormone, amylin, is a feature of type 2 diabetes. Amylin aggregation has been associated with cerebrovascular dysfunction and brain accumulation of HIF1 α and HIF2 α in patients with Alzheimer's Disease, with possible consequences for mitochondrial respiratory function and tissue energetics. This talk will consider the inter-relation of hypoxia, oxygen-sensing pathways and energy metabolism in the brain from these differing perspectives.

SA48

A study of hypoxia and innate immune mechanisms in experimental autoimmune optic neuritis

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Introduction: Optic neuritis is an inflammatory demyelinating (i.e. loss of insulation around nerve fibres) disease of the optic nerve, and it is a common presenting symptom in patients with multiple sclerosis (MS). The visual deficits caused by acute optic neuritis, which can include blindness, often appear to resolve over time, but permanent residual deficits typically remain due to unresolved demyelination and to axonal degeneration. The acute deficits are typically attributed to the demyelination, but more recent evidence raises the possibility that the deficits could be due to inflammatory hypoxia. Thus the optic nerve may have insufficient oxygen to support the mitochondrial function essential for impulse activity and to prevent axonal degeneration. We have therefore examined whether the inflamed optic nerve is hypoxic, using an animal model of MS, experimental autoimmune encephalomyelitis (EAE).

Method: EAE was induced in female Dark Agouti rats by immunization with recombinant myelin oligodendrocyte glycoprotein and adjuvant. Control animals were “immunized” with adjuvant only. Tissue hypoxia was assessed using an immunohistochemical probe, pimonidazole, injected intravenously, and by labelling for endogenous hypoxia-inducible factor-1a (HIF1a). Oxidative and nitrative stress was assessed by labelling for inducible nitric oxide synthase (iNOS) and 3-nitrotyrosine (3NT), as well as by the fluorescence products of dihydroethidium (DHE) previously injected intravenously. Vascular density and diameter were also examined histologically.

Results: Out of 57 nerves collected from animals with EAE, 48 exhibited optic neuritis and were identified as EAE with optic neuritis (EAE-ON), while only 9 showed no sign of inflammation and were designated EAE-NON. The inflamed optic nerves (EAE-ON) were found to be hypoxic, with significantly higher labelling for HIF1a ($p=0.002$) and pimonidazole ($p=0.001$) compared with nerves from control animals (IFA, $n=29$ nerves). Hypoxia is known to trigger oxidative and nitrative stress, revealed in our study by significantly higher labelling for iNOS ($p<0.001$) and 3NT ($p=0.003$), and fluorescence due to DHE ($p=0.055$) in the EAE-ON nerves compared with nerves from IFA animals. EAE-ON nerves also showed significant dilation of capillaries compared with IFA ($p<0.001$).

Conclusion: We conclude that the inflamed optic nerve is hypoxic, associated with oxidative and nitrative stress and damage, as well as alterations in the vasculature. This finding has implications for the treatment of optic neuritis, and provides insights on the early detection and treatment of MS.

SA49

Exploring the effect of hypoxia (1% O₂) on the neuroinflammatory activity of mouse primary microglial cells and microglial cell line (BV-2 cells) under Lipopolysaccharide (LPS) induced inflammatory stimulation.

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Cerebral hypoxia is a feature for several neurological diseases. The low concentration of oxygen and existing inflammation in the brain in these diseases forms a vicious circle that damages brain cells and inhibits the brain regeneration. Neuroinflammation is thought to potentially play both a positive and negative role in hypoxic injury since, depending on severity and duration of the pro-inflammatory stimuli, with prolonged stimulation being associated with activation of cell death pathways and the promotion of damaging oedema and systemic immune invasion. As such, better understanding the ways in which hypoxic stimulation alters the inflammatory activity of microglial cells (the specialised form of immune cells unique to the CNS) is vital to understanding and exploiting endogenous neuroinflammation during hypoxic injury.

To investigate the role of hypoxia in neuroinflammation we established a model of microglial inflammatory stimulation, using a dosage curve of Lipopolysaccharide (LPS) from 1ng/mL to 10,000ng/mL, which were applied to both mouse primary microglial cells and a microglial cell line - BV-2 cells in either normoxia (21% O₂) and hypoxia (1% O₂) oxygen exposures, for 24h. Responses were measured in terms of cell viability, cellular death, TNF- α production, and inflammatory gene expression, as well as phagocytosis through immunofluorescence imaging. Further exploration of the role of established cellular hypoxic response pathways, specifically the role of the Hypoxia Inducible Factors (HIF) 1 and 2, was then conducted by observing how the established cellular responses altered by co-exposure of the cells to 100uM of a clinically approved 100uM PHD2 inhibitor, namely FG4592 (Roxadustat).

We found that BV-2 cells generally showed decreased inflammatory signalling, i.e. TNF- α production in response to hypoxia regardless of LPS dosage, but also that hypoxia did not induce inflammatory signalling in the absence of the LPS (Figure 1). No consistent effect for FG4592 on TNF- α production was identified during hypoxia, however, it did both non-significantly reduce TNF- α expression for all LPS dosages in normoxia and ameliorate hypoxia induced cell death (Figure 2). Mouse Primary microglia displayed a more varied response. Hypoxia generated an apparent increase in cell death provided LPS stimulation was present (Figure 3), unlike in BV-2 cells where hypoxic death was not dependent on LPS stimulation, and it only appeared to promote existing inflammatory signalling at low levels of LPS stimulation (10ng/mL + 1ng/mL), which may suggest hypoxia limits the inflammatory activity of already stimulated/activated microglia but acts as an inflammatory promotor itself when such activation would otherwise be limited (Figure 4). Our phagocytosis assay results were inconclusive.

Our findings suggest that hypoxia limits inflammatory activity by BV-2 cells but may promote it in primary microglia when total inflammatory stimulation is limited. Additionally, inflammatory

stimulation seemingly makes primary microglia, though not BV-2s, more susceptible to hypoxic cell death. FG4592 was effective in ameliorating hypoxic cell death and metabolism loss, seemingly through inflammation independent mechanisms in BV-2 cells (Figure 5.)

Figure 3: Bar chart displaying MTT activity for BV-2 cells under hypoxic and LPS stimulation. Results are displayed as % of normoxic controls \pm SD. Significances are displayed above bars for differing FG4592 dosage of same oxygenation (21% Oxygen = *, 1% Oxygen = #) (* = $p < .05$, ** = $p < .005$, *** = $p \leq .001$)

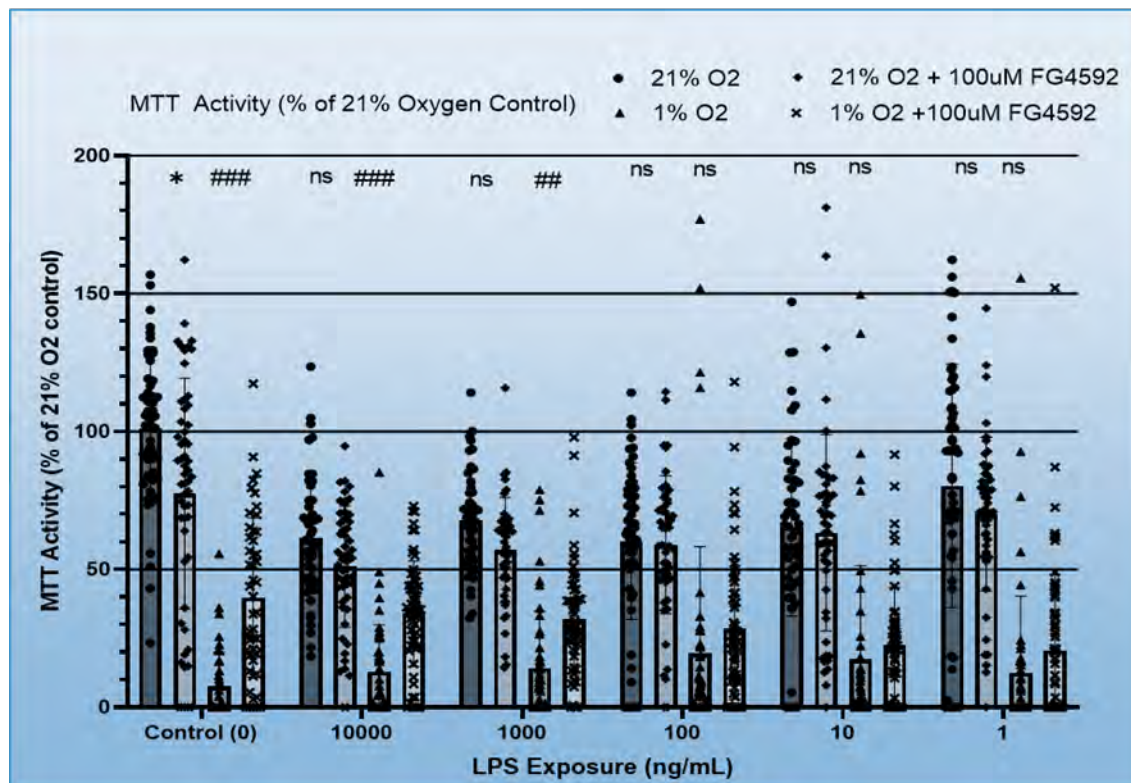


Figure 4: Bar chart displaying TNF- α expression for Mouse Primary Microglia under hypoxic and LPS stimulation. Results are displayed as pg/mL of TNF- α \pm SD.

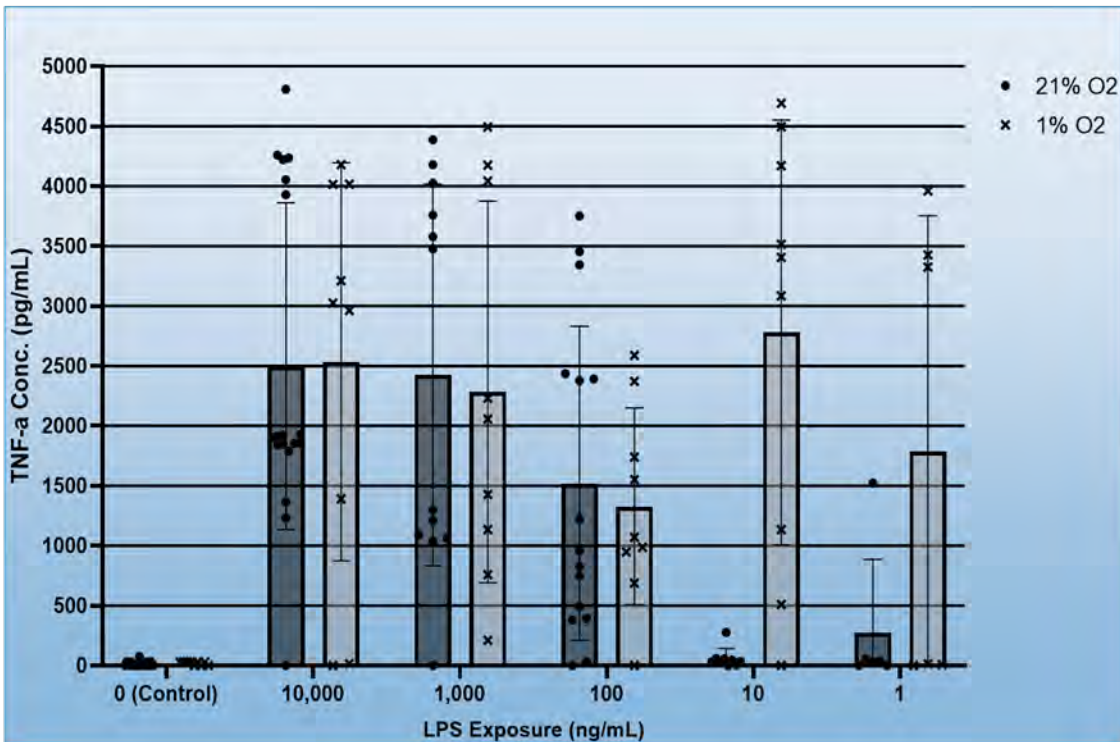


Figure 1: Bar chart displaying TNF- α expression for BV-2 cells under hypoxic and LPS stimulation. Results are displayed as pg/mL of TNF- α \pm SD. Significance is displayed above bars for differing FG4592 dosage of Same oxygenation and LPS dosage (21% Oxygen = * , 1% Oxygen = #) (* = $p < .05$, ** = $p < .005$, *** = $p \leq .001$)

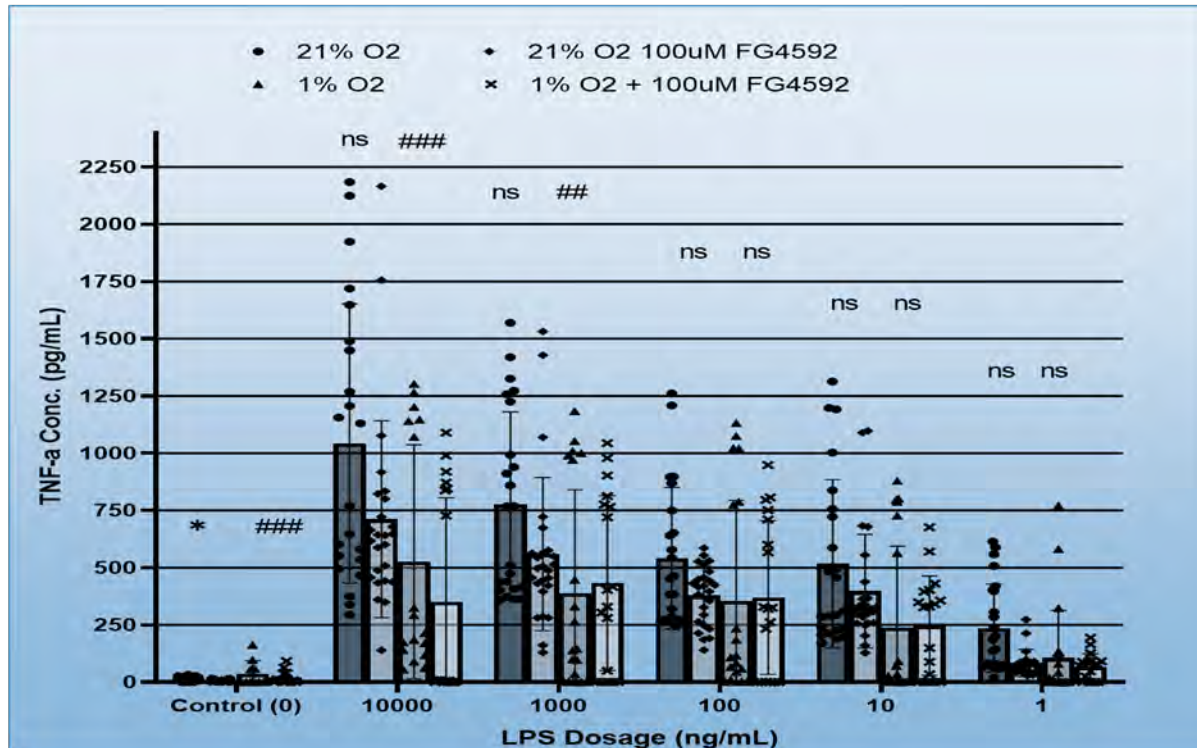


Figure 5: Bar chart displaying LDH concentrations for Mouse primary microglia under hypoxic and LPS stimulation. Results are displayed as % of normoxic maximum release \pm SD

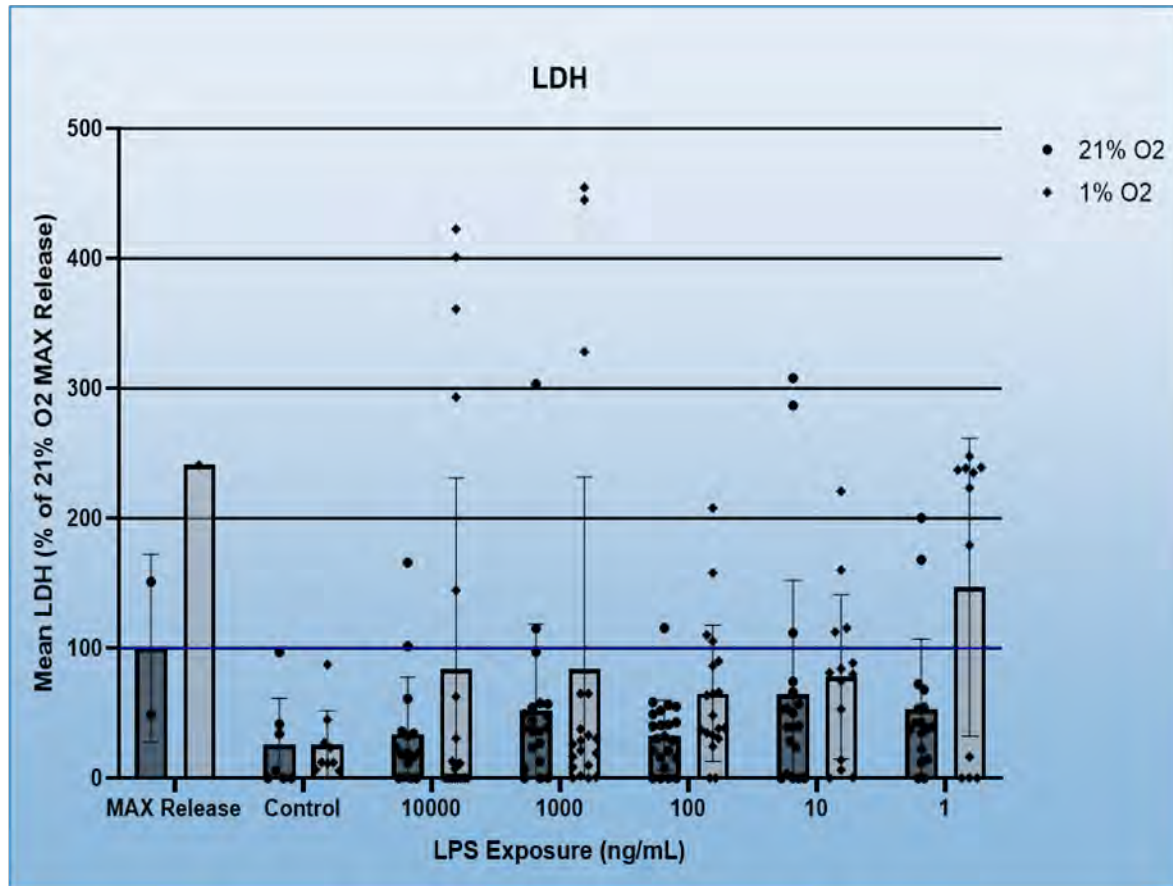
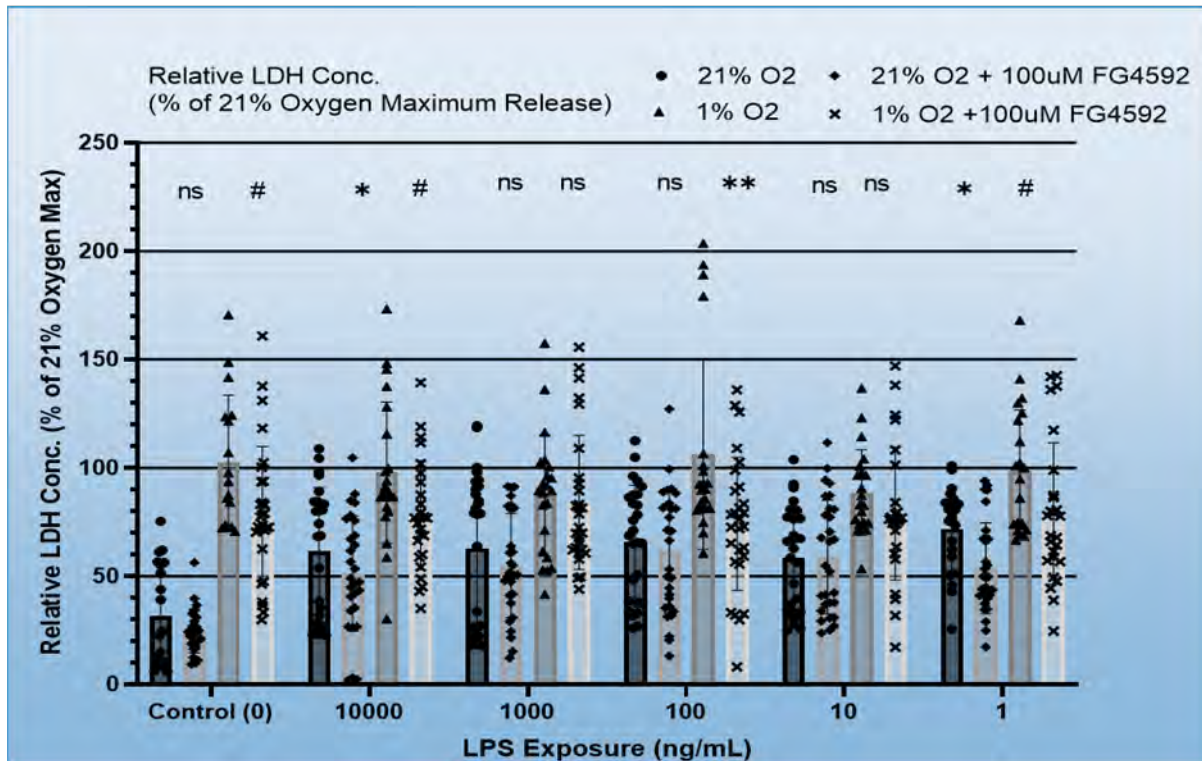


Figure 2: Bar chart displaying LDH concentrations for BV-2 cells under hypoxic and LPS stimulation. Results are displayed as % of normoxic maximum release \pm SD. Significances are displayed above bars for differing FG4592 dosage of same oxygenation (21% Oxygen = *, 1% Oxygen = #) (* = $p < .05$, ** = $p < .005$, *** = $p \leq .001$)



SA50

Hypoxia signalling in vascular dementia.

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Introduction: Vascular cognitive impairment (VCI) refers to cognitive decline attributed to vascular risk factors. Vascular dysfunction produces cerebral hypoperfusion and the brain uses the hypoxia inducible factor (HIF) pathway to compensate and restore oxygen availability. White matter is vulnerable to hypoxia, and an emerging area of interest aims to understand whether epigenetic processes contribute to this. A handful of reports showed that manipulating small, non-coding microRNAs (miRNAs) may provide new therapeutic strategies in animal models of VCI. The aim of this study was to use a hypothesis free approach to profile miRNA changes in the white matter of a mouse model.

Methods: Twenty male C57BL6 mice were randomised to undergo bilateral carotid artery stenosis (BCAS) or sham surgery. This involved wrapping microcoils with 180 or 500µm diameters, respectively, around both carotid arteries. Subsequently, RNA was harvested from the white matter, hippocampus, and cortex at either 7 or 30d post-surgery and electrophysiology was performed on the optic nerves. RNA was sequenced and bioinformatics analysis was performed (Genewiz, UK). A false discovery rate of <0.05 was applied to the bioinformatics analysis to confirm differentially expressed miRNAs (DEMs). Electrophysiology data, compound action potentials (CAPs) are presented as mean ± standard error and unpaired t-tests or mixed two-way repeated measures ANOVAs were used.

Results: At 7 and 30d post surgery, all the sham optic nerves consistently displayed a third peak in the recorded CAP, but most of the BCAS nerves did not. There was also differential expression of several miRNAs following BCAS in the hippocampus and white matter at both timepoints. The greatest number of DEMs (76) were observed in the hippocampus, and 45 survived multiple comparisons. There were 10 and 29 DEMs in the white matter at 7 and 30d, respectively, and mmu-let-7K, miR-30d-5p, and mmu8-miR-362-5p all survived multiple comparisons. Pathway analysis suggests the genes regulated by these DEMs are associated with cell signalling and axon guidance.

Conclusions: The variability in the optic nerve function after BCAS suggests that hypoperfusion may impact white matter most via disruption to the smallest diameters axons. Our data driven approach identified several miRNAs that are differentially regulated in the white matter following hypoxia. While further investigation is required to characterise the roles of these miRNAs, these results suggest epigenetic processes are involved, and we hope this could eventually lead to new therapeutic targets in the future.

SA51

The Role of ATP-Sensitive Potassium Channels in Migraine Pathogenesis

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Migraine, a prevalent and debilitating neurological disorder, remains a puzzle with elusive pathophysiological mechanisms. Recent investigations have shed light on the involvement of intracellular cyclic adenosine monophosphate (cAMP) in migraine pathogenesis, implicating cAMP-dependent signaling pathways in migraine attacks. These studies revealed that oral administration of cilostazol, a cAMP degradation blocker, induced migraine attacks in individuals with migraine, suggesting a pivotal role of cAMP accumulation in migraine onset.

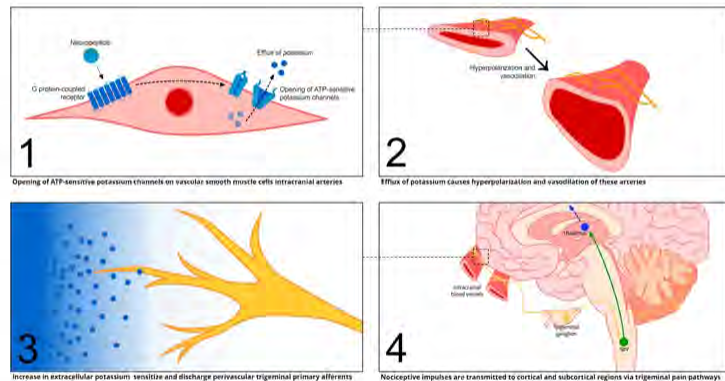
Further exploration led to the hypothesis that downstream effects of cAMP-mediated migraine attacks involve the opening of potassium channels. Provocation studies conducted in our laboratory demonstrated that openers of adenosine-triphosphate (ATP)-sensitive potassium channels and large conductance calcium-activated potassium channels triggered migraine attacks in individuals with migraine.

This lecture presents an overview of our research, emphasizing the pivotal role of ATP-sensitive potassium channels in migraine pathogenesis. Understanding the involvement of these channels offers promising avenues for targeted therapeutic interventions and deeper insights into the complex mechanisms underlying migraine.

A Proposed Trigemino-vascular Ion Channel Hypothesis of Migraine Pathogenesis

A plausible explanation of migraine pathogenesis suggests the following sequence of events within the framework of the trigemino-vascular system: 1) various signaling molecules (e.g. nitric oxide, calcitonin gene-related peptide, pituitary adenylate cyclase activating peptide) initiate a cascade of intracellular processes that result in opening of ATP-sensitive potassium channels on vascular smooth muscle cells within the intracranial arteries 2) efflux of potassium causes hyperpolarization and vasodilation of these arteries 3) increase in extracellular potassium provides the requisite electrochemical gradient to sensitize and discharge perivascular trigeminal primary afferents in the walls of intracranial arteries, 4) nociceptive impulses are transmitted to and processed by cortical and subcortical regions via ascending trigeminal pain pathways, ultimately resulting in the perception of migraine pain. Of note, this line of reasoning emphasizes that elevations in extracellular levels of positively charged ions, not potassium exclusively, may be the principal drivers needed to activate and sensitize trigeminal primary afferents in the walls of intracranial arteries.

• Supplement to: Ashina M. Migraine. *N Engl J Med* 2020;383:1866-76. DOI: 10.1056/NEJMra1915327



<https://bit.ly/49GUVo1>

Key references: 1: Ashina M. Migraine. *N Engl J Med*. 2020 Nov 5;383(19):1866-1876. <https://doi.org/10.1056/nejmra1915327> 2: Ashina M, Hansen JM, Do TP, Melo-Carrillo A, Burstein R, Moskowitz MA. Migraine and the trigemino-vascular system-40 years and counting. *Lancet Neurol*. 2019 Aug;18(8):795-804. [https://doi.org/10.1016/s1474-4422\(19\)30185-1](https://doi.org/10.1016/s1474-4422(19)30185-1)

SA52

New insights to KATP cardiovascular and skeletal muscle channelopathies

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ATP-sensitive potassium (KATP) channels are expressed throughout the body and serve to couple cellular metabolism to membrane excitability. The channels are composed of pore-forming Kir6 subunits co-assembled with obligate regulatory SUR subunits and are critically regulated by the intracellular nucleotides ATP and ADP - activating in response to metabolic compromise. Mutations of the *KCNJ11* and *ABCC8* genes, which encode the predominant Kir6.2 and SUR1 subunits expressed in pancreatic beta-cells, have long been associated with insulin secretion disorders. The effects of mutations of the paralogous *KCNJ8* and *ABCC9* genes (encoding Kir6.1 and SUR2), remained less clear, until discoveries over the last decade which have now associated congenital gain-of-function with the rare heritable disorder Cantu Syndrome, and loss-of-function with *ABCC9*-related intellectual disability and myopathy syndrome (AIMS). Here we report a summary of recent works dissecting the mechanisms of cardiovascular remodeling in Cantu Syndrome using knock-in mouse models of the disease, which reveal that KATP overactivity in vascular smooth muscle (VSM) drives low systemic vascular resistance, and secondary cardiac remodeling to high-output heart failure (HOHF). Inspired by these findings, we will report preliminary data testing the generality of this pathophysiological cascade, derived from the conditional deletion of other ion channels in mice, which tests whether VSM hypo-excitability inevitably drives HOHF. In addition, we will summarize recent advances in understanding of the human consequences of LoF mutations in *ABCC9* in AIMS, alongside dissection of skeletal muscle and cardiac pathology, and preliminary data on novel gene-therapy approaches to counter myopathy.

SA53

Impaired electro-metabolic signaling in capillary pericytes disrupts hemodynamic control in aging and Alzheimer's disease

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Disruptions to the mechanisms governing the metabolic supply-and-demand relationship that are integral to brain energy homeostasis can have deleterious consequences on brain health, making it vulnerable to the development of age-related disorders like Alzheimer's disease (AD). Energy supply is dynamically and precisely controlled by mechanisms inherent to the cerebral vasculature, and here, capillary thin-strand pericytes are quickly emerging as key players. A K_{ATP} channel signalling complex imbues pericytes with the ability to detect subtle changes in local glucose and rapidly couples decreases in this key substrate to robust increases in blood flow. Blocking glucose import into the brain with a glucose transporter-1 blocker (1 μ M BAY-876) activates pericyte K_{ATP} channels to increase brain blood flow in young (2-4 months) mice. Remarkably, this effect is completely lost in older (12-15 months) mice, and even earlier (6-8 months) in mice with familial AD mutations. Our emerging data suggest that a key interaction between membrane cholesterol and K_{ATP} channels underlies this dysfunction, and disrupting cholesterol in older mice reinstates the ability of pericyte K_{ATP} channels to sense decreases in local glucose. Perturbations in pericyte membrane cholesterol due to aging or AD may disrupt the delicate macromolecular organization of K_{ATP} channels, causing a breakdown of energy sensing mechanisms and progressive loss of hemodynamic control by pericytes.