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Book of Abstracts

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Keynote Lecture

Neuronally differentiated macula densa cells control interoception, organ crosstalk, and physiological tissue regeneration

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Commonly used transcriptomics technologies have provided great insight into gene expression, but only a static snapshot of the dynamic processes that define life. Physiology is key to a deeper understanding of these dynamic biological processes, organ function, disease pathogenesis, and (patho) physiological mechanistic insights are absolutely required to take the next steps in therapeutic development. We developed serial intravital imaging approaches using multiphoton microscopy (MPM) of multiple organs to functionally phenotype intact living tissues in vivo at single-cell resolution and to guide human translation. Unbiased MPM imaging of the kidney, a major regulatory organ of body homeostasis, uncovered the key sensory and regulatory functions and neuronal differentiation of the understudied minority cell type of the macula densa (MD). Calcium imaging revealed that MD cells are the nephron's autonomous pacemaker and play central and direct roles in local intrarenal and systemic interoception and brain/gut/endocrine-kidney crosstalk via MD Avpr1a for vasopressin-mediated osmosensing, via Cckbr for gastrin/cholecystokinin-mediated postprandial hyperfiltration, and via Gcgr for glucagon-mediated protein/NO metabolism. Serial MPM imaging of single mesenchymal and endothelial progenitor cells with genetic cell-fate tracking in the same tissue volume, performed daily over two weeks, identified the MD cell epicenter and pattern of cell recruitment and tissue remodeling, especially under conditions of loss of body fluid and salt. The analysis of the MD cell molecular fingerprint confirmed their neuronal differentiation, including high expression of numerous neurodegenerative disease risk genes and secretion of angiogenic, tissue growth and patterning, and extracellular matrix remodeling factors. These studies painted a much more complex picture of MD cell functions than previously thought and illustrate how neuronally differentiated sensory and regulatory cell types in many organs can be a key to improved understanding of physiological tissue regeneration and therapeutic targeting.

Mammalian generation of cyanide: mechanism, action, physiological and pathophysiological roles

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Introduction. Cyanide has long been regarded as a potent cytotoxin, acting primarily through inhibition of mitochondrial Complex IV (cytochrome c oxidase). However, recent findings reveal a more nuanced picture. At low concentrations (nanomolar to low micromolar), cyanide stimulates Complex IV activity, enhances ATP production, and promotes cell proliferation; only at higher levels (high micromolar to millimolar) does it exert the well-established toxic effects. Emerging evidence points to an endogenous role of cyanide in mammalian biology. Low levels of cyanide are generated from glycine in lysosomes, a process requiring acidic pH and peroxidase activity.

Aims. To overview of the biogenesis and functional role of cyanide in mammals.

Methods. Overview and synthesis of the relevant peer-reviewed scientific literature and additional unpublished data.

Results. Cyanide meets many of the criteria for classification as a gasotransmitter, sharing features with NO, CO, and H_2S. It modifies proteins through S-cyanylation, influences mitochondrial bioenergetics, and, in low concentration, protects cells against hypoxic injury. Low levels of cyanide regulate cell metabolism and cell proliferation in hepatocytes and endothelial cells. Excessive cyanide production—as in nonketotic hyperglycinemia cells, which have a genetic deficiency to degrade glycine and as a consequence accumulate high levels of cyanide, proves deleterious. Similarly, in Down syndrome, cyanide emerges as a significant contributor to metabolic dysfunction. This overproduction is linked to reduced expression of thiosulfate sulfurtransferase, a key cyanide-detoxifying enzyme.

Discussion. Cyanide emerges as the fourth gaseous transmitter, with regulatory roles in health and disease. Targeting the cyanide pathway may thus offer multiple opportunities towards modulation of human metabolism and cell fate.

Randi E et al (2021) Proc Natl Acad Sci USA. 118:e2026245118.
Zuhra K and Szabo C (2022) FEBS J. 289:2481-2515
Zuhra K et al (2025) Nat Metab 7:531-555.
Petrosino M et al (2025) Neurotherapeutics 22:e00719.
Kieronska-Rudek et al (2026) Exp Biol Med 251: 10856

Astrocytes: Master regulators of brain function and disease

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Astrocytes are essential for the proper functioning of the vertebrate brain. Unlike neurons, they are electrically non-excitable cells, meaning they do not generate fast regenerative electrical signals. Instead, they rely on dynamic changes in the concentration of ions inside the cell, of which changes in Na^+, pH and Ca^{2+}, are the best studied. These glial ion transients, particularly Ca^{2+} signals, play a key role in the interaction between astrocytes and neurons at synapses, as well as in their communication at the neurovascular unit. Furthermore, astrocytes play a crucial role in maintaining ionic homeostasis in the extracellular space. This involves the uptake of extracellular potassium (K^+) by Kir4.1 channels and the Na^+/K^+-ATPase (NKA). Control of extracellular K^+ is a central mechanism by which astrocytes regulate neuronal excitability and network performance. In addition to its well-known role in extracellular K^+ homeostasis, the NKA is also the dominant mechanism for exporting Na^+ from astrocytes, thereby establishing a low intracellular Na^+ concentration against a high inward Na^+ gradient. The latter provides the driving force for a multitude of other plasma membrane transporters including $Na^+/K^+/2Cl^-$ cotransporters (NKCC1), Na^+/H^+ exchangers (NHE), and Na^+-dependent glutamate transporters (EAATs). These challenge the Na^+ gradient both at rest and during neuronal activity, making a low intracellular Na^+ concentration and NKA activity the “core and hub” of astrocyte function. In my talk, I will provide an overview of the essential functions of astrocytes and discuss how their impairment contributes to cell damage and associated cell death in disease. Finally, I will present our laboratory's new, unpublished results, revealing unexpected cellular and subcellular heterogeneity in astrocytic Na^+-homeostasis. This results in functionally distinct astrocyte subgroups and subdomains that are optimized for the efficient regulation of ion and transmitter homeostasis in the brain.

Session I.
Nephrology I.: Renal pathophysiology to potential treatment MANET-MÉT

A novel neuronally differentiated endothelial cell subtype regulates organ blood flow in the kidney and brain

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Vascular endothelial cells (ECs) play critical roles in the physiological maintenance of organ blood flow, coordinate vascular repair, angiogenesis, and vascular remodeling, and their organ specific differentiation contributes to organ specific functions. Nitric oxide (NO) is a central mediator of vascular sex dimorphism; greater NO bioavailability in females leads to a more vasodilatory, anti-inflammatory, and adaptive vascular phenotype. Our recent work using Nos1 cell lineage tracing identified a novel neuronally differentiated subtype of endothelial cells – NECs in brain, retina, and kidney resistance arterioles. High-resolution single-cell transcriptome analysis of brain and kidney endothelial cells (ECs) revealed highly NEC-specific expression of pH and mechanosensitive G-protein-coupled receptor 68 (GPR68), along with traditional and novel vasoactive and angiogenic factors (Nos1, Cytl1, ACER2). Importantly, estrogen receptor 1 (ESR1) and G-protein-coupled estrogen receptor (GPER) are highly enriched in NECs compared to other ECs. To specifically test the functional role of NEC Nos1 in brain and kidney arterioles in vivo, we performed silencing of NEC Nos1 gene expression in vivo using iv injections of $2X10^{11}$ GC/kg endothelial targeting AAV2-QUADYF serotype containing either control GFP RNA (NEC-WT), Nos1 shRNA (NEC-Nos1 knockdown (KD)). Histological analysis of endothelium-specific GFP labeling and Nos1 RNAscope confirmed and validated the EC/NEC-specific targeting approach and highly efficient NEC Nos1 (76%) gene silencing. In vivo MPM imaging of intact living mouse kidney and brain of Nos1-KD animals revealed severe, spasm-like vasoconstrictions of resistance arterioles (20.2+/-7 vs. 29.1+/-8 um, $p<0.001$), reduced capillary density (19.9+/-0.2 vs. 22.6+/-0.05 um3/unit volume, $p<0.001$), impaired vascular barrier function (albumin leakage), and reduced regional blood flow (12 +/-4 vs. 70+/-12 nL/s, $p<0.001$) compared to wild type (n=4 each). Importantly, acute volume expansion (10% of total blood volume administered as a slow intravenous PBS bolus) induced a rapid rise in renal blood flow in control animals, whereas this response was abolished in NEC-Nos1-KD mice (n=4). These results suggest that NECs may represent an intrinsic estrogen-dependent vasoprotective mechanism. GPR68-dependent mechano- and metabolic sensing, and NOS1-derived nitric oxide signaling, control rapid hemodynamic responses, whereas long-term vascular remodeling responses are controlled via ACER2–S1P and Cytl1 to preserve vascular stability and metabolic–vascular homeostasis.

Methane improves graft function in a porcine kidney transplantation model

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Keywords: kidney transplantation, ischemia-reperfusion, mitochondria

Kidney transplantation is the most effective treatment for end-stage renal disease; however, graft function and survival are significantly compromised by the inflammatory response following ischemia-reperfusion injury. Methane (CH_4) possesses antioxidant, anti-inflammatory, and mitochondrial-stabilizing properties, making it a potential organ-protective agent. Our study aimed to investigate the effects of CH_4-enriched histidine-tryptophan-ketoglutarate (HTK-CH_4) preservation solution in a porcine model of autotransplantation during static cold storage.

Left nephrectomy was performed in anesthetized, ventilated pigs (license number: V./2710/2024). The kidneys were perfused and stored for 16 hours at 0-4 °C in either standard HTK (Custodiol, Dr Franz Koehler Chemie, Germany) or HTK-CH_4 solution (1 L HTK solution enriched with 25 L, 2.2% CH_4-air mixture, n=5 per group). Following autotransplantation, the contralateral kidney was removed. Systemic hemodynamics, renal artery flow (RAF; TTFM-2, Hugo Sachs Elektronik, Germany), hourly diuresis, serum creatinine levels were monitored for 24 hours. Inflammatory activity in the renal tissue was characterized by xanthine oxidase (XOR) and myeloperoxidase (MPO) enzyme activities, while mitochondrial function in the renal cortex and medulla was assessed via high-resolution respirometry (Oxygraph 2K, Oroboros Instruments, Austria). Further renal tissue samples were collected for histological evaluation.

No significant differences were observed in mean arterial pressure or cardiac output between the two groups. In the HTK-CH_4 group, higher RAF values and lower creatinine levels (4.01 ± 0.2 vs. 4.7 ± 0.3 mg/dl) were recorded, indicating improved renal function. The HTK group required more frequent diuretic administration. Serum creatinine levels were reduced in the HTK-CH_4 group (248 ± 31 µmol/L vs. 315 ± 42 µmol/L), and tissue MPO and XOR activities, as well as plasma MPO levels were decreased. Histological examination revealed structural damage, which was attenuated in the HTK-CH_4 group. The cytochrome c test demonstrated a lower respiratory rate increase ($p = 0.023$) in the HTK-CH_4 group, indicating superior integrity of the outer mitochondrial membrane.

Methane-enriched preservation solution effectively attenuates oxidative and inflammatory damage caused by ischemia-reperfusion, thereby improving early functional recovery of the graft. Methane represents a promising, cost-effective bioactive component for clinical organ preservation protocols.

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Kidneys of heterozygous dihydrolipoamide dehydrogenase KO mice are partially protected from ischemia-reperfusion injury

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Keywords: mitochondria, reperfusion injury, reactive oxygen species

Introduction: Succinate is produced by the 2-oxoglutarate dehydrogenase complex (OGDHc), one of the rate-limiting steps of the citric acid cycle, and then by succinyl-CoA synthetase. During ischemia, the reverse mode operation of succinate dehydrogenase (Complex II) produces succinate from fumarate, leading to succinate accumulation. When O_2 supply is restored, succinate is rapidly oxidized, generating a large amount of reactive oxygen species (ROS), which is the main cause of reperfusion injury. Decreased OGDHc activity can reduce succinate and fumarate productions. Therefore, we investigated whether heterozygous deletion of dihydrolipoamide dehydrogenase (DLD), the E3 component of OGDHc, could provide protection against kidney ischemia-reperfusion injury (IRI) in mice.

Methods: We applied unilateral 15-minute renal ischemia or sham surgery (SO) in wild-type (WT) and $DLD^{+/-}$ male mice (with contralateral nephrectomy), followed by 24 hours of reperfusion. Renal function was assessed based on blood urea nitrogen (BUN) levels and injury biomarkers such as kidney injury molecule-1 (KIM-1), lipocalin-2 (LCN2), heme oxygenase 1 (HMOX-1), and hypoxia-inducible factor 1α (HIF-1α) mRNA expression, and acute tubular necrosis (ATN) was scored.

Results: Mitochondria isolated from IRI kidneys of $DLD^{+/-}$ mice showed reduced OGDH activity, ROS production and functional protection against IRI. Compared to SO mice, BUN and ATN score increased less in $DLD^{+/-}$ than in WT mice after renal IRI. Based on the lower LCN2 mRNA expression, the outer stripe of the medulla was less damaged in $DLD^{+/-}$ mice than in WT mice, while the marker of proximal tubule injury, KIM-1 mRNA expression, increased similarly in $DLD^{+/-}$ and WT mice after IRI. A tendency (P = 0.068) of increased mitochondrial biogenesis was detected in $DLD^{+/-}$ compared to WT mice after IRI. However, the changes in mitochondrial fission and fusion, as well as HMOX-1, HIF-1α and some the inflammatory mRNA markers did not differ between $DLD^{+/-}$ and WT mice.

Conclusion: $DLD^{+/-}$ mice are partially protected against renal ischemia-reperfusion injury. The results raise the possibility that the renal medulla is protected, but the cortex is not or less protected in $DLD^{+/-}$ mice. Longer reperfusion may better reveal the protection provided by $DLD^{+/-}$ due to the reduced ROS production and better-preserved mitochondrial function.

PDE5 inhibition reduces diabetic tubular damage

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Keywords: diabetes, fibrosis, tubules, cGMP, phosphodiesterase

Diabetes (DM) is the leading etiology of chronic kidney disease with increasing incidence worldwide. Diabetic kidney disease is characterized by glomerular and tubular dysfunction and enhanced PDE5 activity with reduced cyclic guanosine monophosphate (cGMP) levels in the kidneys. We previously demonstrated that PDE5 inhibition restores renal cGMP levels in rats with T1DM and reduces podocyte damage. However, the effects of PDE5 inhibition on high glucose induced tubular dysfunction is still unclear. Thus, we aimed to investigate the tubular effects of selective PDE5 inhibitor vardenafil (V) treatment *in vivo* and *in vitro*.

Zucker diabetic fatty (ZDF) rats (n=15) and lean controls (CTL, n=7) were used for 25 weeks follow-up, starting at the age of 7 weeks. 8 ZDF rats received V treatment (ZDF+V) at the beginning of the study (10 mg/kg body weight daily) with continuous dose adjustments. At the age of 32 weeks, the study was terminated and kidneys were harvested for histology, immunohistochemistry and molecular analyses. Human HK-2 proximal tubule cells were challenged with PBS (CTL) or 30 mM glucose (HG) with or without 50 μM V for 48h then immunocytochemistry and gene expression analysis was performed. Statistical significance was evaluated with Kruskal-Wallis test or ANOVA.

Compared to CTL, diabetes induced 3-fold renal mRNA over-expression of tubular injury marker lipocalin-2 *(Lcn2)* in ZDF, but V treatment reduced *Lcn2* to CTL levels ($p<0.001$). Accordingly, renal histology depicted significant tubular dilatation and interstitial extracellular matrix deposition in non-treated ZDF rats, reduced by 25% in ZDF+V ($p<0.05$). In ZDF we also observed 2-fold and 3-fold over-expression of *Acta2* and *Col1a1* mRNA, respectively, accompanied by increased *Tgfb1* expression, all attenuated by V treatment ($p<0.05$). In vitro, HG treated HK-2 cells depicted reduced cGMP immunoreactivity as compared to CTL cells, which was normalized by V treatment. Gene expression of *LCN2, TGFB1* and *COL1A1* were significantly induced in HG cells, but remained at CTL levels in HG+V cells ($p<0.05$). Interestingly, HG did not induce *ACTA2*, but V lowered its expression by 40% ($p<0.001$).

In summary, our data suggest that chronic vardenafil administration can significantly reduce diabetes induced renal tubular dysfunction and prevent the progression of tubulointerstitial fibrosis.

Sigma-1 receptor as a novel therapeutic target in diabetic kidney disease

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Keywords: Sigma-1 receptor, diabetic kidney disease, fibrosis

Background and aims: Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease. Current treatments cannot halt the progression of renal injury, highlighting an urgent need for therapies targeting key disease mechanisms. Previously, we described the protective role of Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) in acute kidney injury. Thus, we hypothesized a similar protective effect in DKD.

Methods: Diabetes mellitus was induced in male Wistar rats using streptozotocin, followed by a seven-week FLU treatment. Metabolic and renal parameters were assessed along with a histological analysis of glomerular damage (PAS) and tubulointerstitial fibrosis (Masson's trichrome). The effects of inflammation and hypoxia were tested in lipopolysaccharide (LPS)- or hypoxia-induced human proximal tubular cells (HK-2). Cells were treated with FLU. Inflammatory and hypoxia markers were measured by RT-qPCR.

Results: FLU improved renal function and urinary biomarkers of tubular damage (KIM-1, NGAL) in DKD rats. In parallel, FLU reduced glomerular damage and fibrosis. FLU decreased LPS-induced TLR2, NFKB1, and IL6 expressions, as well as hypoxia-induced HIF1A, SLC2A1, VEGFA, and TGFB1 elevation in HK-2 cells.

Conclusions: S1R activation can slow DKD progression by modulating critical inflammatory, hypoxic, and fibrotic pathways. Thus, S1R may serve as a promising novel therapeutic target in DKD.

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TIMP1 is a key regulator of diabetic nephropathy progression

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Keywords: diabetic nephropathy, disease progression, transcription factors

Diabetic kidney disease (DKD) represents a principal cause of end-stage renal failure and is characterized by progressive renal fibrosis and inflammation. We previously identified tissue inhibitor of metalloproteinase-1 (TIMP-1) as a key mediators of kidney fibrosis in murine models exhibiting variable disease progression, as well as in human focal segmental glomerulosclerosis (FSGS). However, the specific role of TIMP-1 in DKD progression remain insufficiently elucidated.

Our aim was to investigate the TIMP-1-driven molecular mechanisms underlying DKD, type 1 diabetes was induced via streptozotocin administration in TIMP-1-deficient (Timp-/-) and wild-type C57Bl/6J male mice, which were subsequently compared with non-diabetic controls. After eight weeks, serum, urine, and renal tissue samples were collected for analysis. Statistical significance ($p<0.05$) was determined using ANOVA and Kruskal–Wallis tests.

Deficiency of functional TIMP-1 significantly attenuated diabetes-induced glomerulosclerosis and tubular injury, while normalizing serum urea and creatinine concentrations as well as proteinuria levels, despite comparable hyperglycemia and weight loss relative to wild-type diabetic mice. Notably, kidneys from diabetic Timp-/- mice exhibited reduced mRNA expression of *Ctgf*, *Col1a1*, *Col3a1*, and *Lcn2*, approaching levels observed in non-diabetic controls. Furthermore, the absence of TIMP-1 suppressed diabetes-induced activation of the transcription factors EGR1, EGR2, and STAT3.

We can conclude that TIMP-1 deficiency mitigates the pathogenesis of DKD by attenuating fibrosis and renal dysfunction. These findings implicate TIMP-1 as a potential therapeutic target in DKD.

Session II.
TDK (in Hungarian)

Hypercholesterolemia-induced lipidomic alterations in the hearts of male and female rats

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Introduction: Hypercholesterolemia is a major cardiovascular risk factor in both sexes. Our previous results demonstrated that a cholesterol-enriched diet induces diastolic dysfunction in male rats, whereas no significant functional impairment was observed in females.

Aims: Our aim was to investigate whether hypercholesterolemia induces lipidomic alterations in the heart and, if so, whether sex-related differences exist.

Methods: In our earlier experiment, 8-week-old male (n=9) and female (n=9) Wistar rats were assigned to groups and fed either a cholesterol-enriched (HChol) or a standard diet (NChol) for 12 weeks. At the end of the experiment, following pressure-volume catheterization, the hearts were excised and the left ventricles were snap-frozen in liquid nitrogen. The samples were then pulverized, and our collaborative partner performed total lipid extraction followed by high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). The raw lipidomic data were processed and statistically analyzed, and the results were expressed as percentages relative to total polar lipids.

Results: The cholesterol-enriched diet induced hypercholesterolemia in both sexes and significantly increased cardiac tissue cholesterol content (male NChol: 10.1±0.19%, HChol: 11.2±0.26%; female NChol: 11.3±0.21%, HChol: 12.7±0.39%). Cardiac cholesteryl ester content increased significantly only in females in response to the diet (0.482±0.06% vs. 0.901±0.08%). Total tissue cardiolipin was significantly higher in both HChol groups compared to controls. Tetralinoleoyl cardiolipin, the most abundant cardiolipin species, was elevated only in males (6.78±0.16% vs. 7.94±0.22%), whereas in females the total amount of other cardiolipin species increased significantly (1.12±0.06% vs. 1.6±0.09%). In males, serum and tissue cholesterol levels, as well as total cardiolipin content, correlated significantly with left ventricular end-diastolic pressure.

Discussion: A cholesterol-enriched diet induces distinct lipidomic alterations in the hearts of male and female rats. These differences may contribute to the sex-related functional disparities observed under hypercholesterolemic conditions.

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Ethical approval number: XV./734/2023.

Inhibition of L-type amino acid transporters (LATs) impairs thermogenesis in human cervical-derived adipocytes

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Brown/beige adipocytes are characterized by high mitochondrial content and elevated expression of uncoupling protein 1 (UCP1). These cells dissipate energy in the form of heat via UCP1-mediated proton leak across the inner mitochondrial membrane, which results in the uncoupling of the respiratory chain from ATP synthesis. Thermogenically active adipocytes utilize increased amounts of nutrients, including glucose, fatty acids, and amino acids.

In response to adrenergic stimulation (modeled by treatment with cell-permeable dibutyryl (db)-cAMP), the expression of L-type amino acid transporters 1 and 2 (LAT1 and LAT2, encoded by *SLC7A5* and *SLC7A8*, respectively), as well as their heterodimeric partner protein 4F2hc (encoded by *SLC3A2)*, was increased in human cervical-derived adipocytes. In our previous study, we demonstrated that silencing of LAT1 expression attenuated thermogenesis in these cells.

The aim of the present study was to investigate the effect of the pharmacological inhibitor of the LAT transporter family, BCH, on thermogenesis in human cervical-derived adipocytes. Stromal vascular fractions, isolated from human cervical adipose tissue biopsies, were differentiated into mature adipocytes over 14 days. Following differentiation, cells were treated with BCH, db-cAMP, or their combination for 10 hours.

The mRNA and protein expression levels of thermogenic genes were determined by RT-qPCR and Western blotting. Oxygen consumption and extracellular acidification rates were assessed using a Seahorse XF Pro Metabolic Analyzer. Amino acid flux of the adipocytes was analyzed by the Metabolomics Core Facility of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen.

Our results showed that pharmacological inhibition of LAT transporters reduced the consumption of branched-chain amino acids (leucine, isoleucine, and valine), as well as arginine, glutamine, tyrosine, serine, proline, and glycine. Furthermore, it decreased etomoxir-resistant respiration, which reflects amino acid utilization. BCH treatment prevented the db-cAMP-induced upregulation of UCP1 and PGC1α expression. Additionally, UCP1-dependent proton leak respiration was also reduced in the presence of BCH during adrenergic stimulation in human cervical-derived adipocytes.

Our findings suggest that amino acid uptake mediated by LAT transporters is essential for the efficient induction and maintenance of thermogenesis in human adipocytes, and its enhancement may represent a safe therapeutic strategy for the treatment of obesity.

Supervisors: Dr. Endre Károly Kristóf and Dr. Rini Arianti

How does TRPV1 contribute to the regulation of body temperature responses during physical exercise?

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Keywords: trpv1, thermoregulation, exercise

Previous studies suggest that proton-mediated activation of transient receptor potential vanilloid-1 (TRPV1) ion channels contributes to the prevention of excessive increases in body temperature. During physical exertion and acidosis, this activation may be enhanced, thereby helping to limit excessive hyperthermia and the associated decline in performance; however, the effect of TRPV1 deficiency on thermoregulation during physical activity remains unknown. In the present study, following a preconditioning period, exercise capacity was assessed in wild-type (WT) and TRPV1 knockout (KO) male mice using a treadmill protocol. Body temperature was measured before and after exercise using a rectal probe, while temperature changes during running were monitored by thermal imaging. Serum lactate levels were determined before and after exertion. Post-exercise rectal temperature was significantly higher in TRPV1 KO animals compared to their WT counterparts. On the third training day, temperatures were 36.9°C in WT versus 37.5°C in KO mice ($p<0.05$), while on the experimental day values were 37.0°C versus 37.4°C, respectively ($p<0.05$). Thermal imaging measurements confirmed that body temperature in TRPV1 KO mice was approximately 1.5°C higher than in controls ($p<0.05$). Acidosis developed following exercise; however, its magnitude did not differ between genotypes. In contrast, TRPV1 KO mice were able to sustain physical activity for a significantly longer duration on the experimental day (24.0±4.0 min in WT vs. 41.5±3.4 min in KO; $p<0.05$). The absence of TRPV1 leads to altered physiological regulation of body temperature during physical exertion. Although the degree of acidosis was comparable between groups, the same acidic stimulus was less effective in preventing the rise in body temperature in TRPV1 KO mice. Despite this, exercise capacity was significantly increased in the KO animals. It is plausible that, in the absence of TRPV1, reduced thermal feedback and diminished nociception result in elevated body temperature failing to trigger the usual behavioral and physiological termination mechanisms, thereby allowing apparently enhanced endurance.

The effect of the Syk tyrosine kinase inhibitor lanraplenib on immune complex-mediated inflammation

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Keywords: immune complex, Arthus reaction, neutrophil, Syk, therapy

Some systemic autoimmune diseases are driven by immune complex-mediated reactions. Many patients do not respond properly to current therapies, which highlights the need for novel therapeutic targets. The Syk tyrosine kinase is essential in the signaling of Fc receptors in neutrophils, which plays a key role in immune complex-mediated autoimmune inflammation.

Our experiments aimed to understand the effect of the selective Syk inhibitor lanraplenib in an in vivo immune complex- and neutrophil-dependent model, the reverse passive Arthus reaction, as well as on the in vitro Fc receptor-mediated responses of neutrophils.

In the Arthus reaction, ovalbumin was injected retroorbitally, which was followed by intradermal injection of anti-ovalbumin antibodies into the ear. Local inflammation and oedema were monitored by Evans blue dye extravasation and ear thickness measurements. Immune cell infiltration and cytokine levels were quantified by flow cytometry and ELISA. Neutrophils were activated on immune complex surface in vitro. Superoxide production was assesed by cytochrome c reduction and cell spreading was detected using phase contrast microscopy. The motility of neutrophils was tested in the Transwell migration assay.

Lanraplenib significantly reduced dye extravasation and ear swelling, and decreased neutrophil and macrophage recruitment, as well as CXCL2 levels. Lanraplenib dose-dependently inhibited neutrophil superoxide production and cell spreading on immune complex surfaces in vitro, while having no effect on their migratory capacity.

Lanraplenib effectively reduced inflammation in vivo, probably due to impaired post-migratory neutrophil responses, which raises the possibility that lanraplenib might be a promising therapeutic option for immune complex-mediated autoimmune diseases.

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Anti-inflammatory and neuroprotective effects of inflammasome inhibitors following contusion spinal cord injury

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Introduction: Following contusion spinal cord injury, severe neuroinflammation develops in the affected area. Activated inflammasomes play a key role in initiating the pro-inflammatory cascade. The aim of our study was to examine what morphological and functional regeneration can be achieved after spinal cord injury by inhibiting the activation of the NLRP3 inflammasome alone, as well as the combined inhibition of NLRP3 and NLRC4 inflammasomes.

Methods: In our experiments, we induced contusion spinal cord injury at the level of the thoracic segment 10 in Sprague–Dawley rats. Some animals were treated for 4 days with a specific NLRP3 inflammasome inhibitor (MCC950), while others received a dual NLRP3 and NLRC4 inhibitor (C75), administered once daily by intraperitoneal injection at a dose of 10 mg/kg. Control groups received either no treatment or only the vehicle of the administered compounds. In our short-term experiments, we assessed the gene expression of inflammatory markers using qPCR, and mapped microglia/macrophage responses by immunohistochemistry. In long-survival groups, we identified intact/regenerated tracts using retrograde labeling, and evaluated gait using video-based motion analysis.

Results: MCC950 and C75 treatment significantly reduced the expression of the pro-inflammatory genes IL-1β, NLRP3, and caspase-1, while C75 additionally decreased NLRC4 expression in the injured region. The density of microglia/macrophage cells was significantly lower in the treated groups at three and seven days post-injury. The number of retrogradely labeled propriospinal and supraspinal neurons was significantly higher in the treated groups. Our morphological analyses revealed substantial neuroprotection, and our functional assessments showed significant motor improvement in both treatment groups.

Discussion: Based on our results, we conclude that inhibition of the NLRP3 inflammasome alone, as well as the combined inhibition of NLRP3 and NLRC4, significantly reduces neuroinflammation following contusion spinal cord injury. In the long term, this was reflected in the preservation of proprio- and supraspinal pathways and in overall neuroprotection.

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Hemokinin-1 as a modulator of thermoregulation

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Keywords: Hemokinin-1, fever, hypothermia, LPS, thermoregulation

Hemokinin-1 (HK1) is a neuropeptide of the tachykinin family known to exert several functions in the central nervous system[1]; however, its role in thermoregulation has not been previously described. Based on its stable expression in the hypothalamus[2]—a key region in temperature regulation—HK1 represents a strong candidate for a regulatory role in thermal homeostasis. Therefore, our study aimed to elucidate its potential involvement in thermoregulatory mechanisms.

Experiments were conducted on adult male HK1 gene-deficient (KO) and C57Bl/6 (wildtype, WT) mice. We compared core temperature responses following cold and heat exposure, as well as after intraperitoneal administration of low (120 µg/kg) and high (5 mg/kg) doses of lipopolysaccharide (LPS). This experimental process was based on a well-known model, our research group used in previous studies[3].

Our findings show that under physiological conditions, there was no difference between KO and WT groups in response to either cold or heat exposure. However, under pathophysiological stress, i.e., systemic inflammation, we found significant differences. In the late phase of the multiphasic febrile response following low-dose LPS, the temperature rise was significantly attenuated in the KO group. Furthermore, in response to high-dose LPS, HK1 deficiency prevented the development of hypothermia.

These results demonstrate that HK1 may have both pyrogenic and cryogenic properties. Further studies are needed to elucidate the precise mechanisms underlying its role in thermoregulatory responses.

The role of metabolic rhythm in contact dermatitis

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Keywords: metabolic rhythm, contact dermatitis, bmal1

Introduction: Circadian rhythms are fundamental adaptive mechanisms that coordinate physiological functions with daily environmental cycles via molecular clocks at the cellular level. The *bmal1* gene is a central component of this system and is indispensable for maintaining circadian rhythmicity. Clock activity is modulated by both the timing and composition of nutrient intake. A high-fat (HF) diet disrupts circadian organization and metabolic homeostasis, whereas time-restricted feeding (TRF) supports circadian alignment. Metabolic changes also influence immune responses. Previous studies have shown that an HF diet worsens contact hypersensitivity–induced dermatitis, while TRF can attenuate this effect.

Objective: This study aimed to examine the interplay between circadian clock function and feeding patterns in subacute dermatitis induced by contact hypersensitivity.

Methods: bmal1 knockout (KO) and wild-type (WT) mice were assigned to three dietary regimens: standard diet ad libitum (control), HF diet ad libitum (HF AL), and HF diet under time-restricted feeding conditions (HF TRF). Animals were maintained on these diets for 4 weeks. Contact hypersensitivity was induced with trinitrochlorobenzene (TNCB). In the acute model, ears were challenged once after sensitization, whereas in the subacute model, three repeated challenges were applied. Inflammation was evaluated by measuring ear thickness. Tissue samples were further analyzed using flow cytometry and histological techniques.

Results: KO mice displayed attenuated inflammatory responses compared to WT mice, including in the subacute model. Neither HF nor TRF significantly influenced inflammation in KO animals.

Conclusion: The presence of the *bmal1* gene is necessary for dietary interventions to affect the inflammatory response.

Session III.
Nephrology II.: Understanding rare kidney diseases MANET-MÉT

Dent disease: genotype–phenotype correlation and alterations of the endocytotic apparatus of the proximal tubule

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Background: Dent disease is a rare X-linked proximal tubulopathy characterized primarily by low molecular weight proteinuria (LMWP). LMWP is most commonly associated with hypercalciuria and nephrocalcinosis. Although initially oligosymptomatic, the disease may progress to end-stage kidney disease over time. Dent disease is most frequently caused by mutations in the *CLCN5* gene (Dent-1), more rarely by mutations in the *OCRL* gene (Dent-2).

Objectives: Our aim was to retrospectively analyze the clinical characteristics of patients with Dent disease treated at the Department of Pediatrics, University of Szeged, and to compare our findings with cases reported in the international literature. Furthermore, aimed to present the histopathological alterations observed in renal biopsy specimens.

Methods: A retrospective analysis of the clinical phenotype of four genetically confirmed patients with Dent disease was performed (three patients with *CLCN5* mutations and one patient with *OCRL* mutation). Genetic testing was performed by using next-generation sequencing. Renal biopsy was performed in two cases, and histopathological evaluation was conducted at the Department of Pathology, University of Szeged.

Results: Significant phenotypic variability was observed among our patients regarding nephrocalcinosis, hypercalciuria, and renal function. In renal biopsy specimens, the absence of the endocytotic apparatus was identified in proximal tubular cells, providing a direct morphological explanation for the clinically observed LMWP.

Conclusions: In agreement with the international literature, patients with Dent disease followed at our center demonstrate marked clinical heterogeneity. The presented morphological alterations provide both structural and functional insight into the pathomechanism of LMWP in Dent disease.

The role of temperature in TMEM260-associated kidney involvement with incomplete penetrance

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Keywords: cystic kidney, expressivity, temperature, kidney failure

Introduction: TMEM260 *O*-mannosyltransferase deficiency has recently been found implicated in Structural Heart Defects and Renal Anomalies (SHDRA) syndrome. In contrast to the typically consistent phenotype of autosomal recessive cystic kidney diseases caused by biallelic loss-of-function variants, kidney involvement associated with loss of the long TMEM260 isoform shows incomplete penetrance.

Methods: Exome sequencing was performed in two families with SHDRA syndrome. Postoperative kidney failure rates were compared between patients with TMEM260-associated and non–TMEM260-associated truncus arteriosus. A CRISPR/Cas9-generated *tmem260* knockout zebrafish model was used to assess phenotypic consequences. The effects of heat stress on TMEM260 expression, intracellular localization and on mutant embryo morphology were evaluated.

Results: Three affected children were compound heterozygous for TMEM260 variants. Extracardiac manifestations—including medullary cystic enlarged kidney and neurological involvement—were observed only in patient F1P1, who lacked long TMEM260 isoform expression, but not in siblings from family F2 with a residual long isoform expression. The *tmem260* knockout zebrafish showed no baseline phenotype or ciliary dysfunction, and TMEM260 localized to the endoplasmic reticulum and nuclear membrane rather than the primary cilium in human cells. Heat stress induced upregulation of TMEM260 in mIMCD3 cells and triggered marked phenotypic abnormalities in knockout zebrafish, including axial deformities, absent cardiac looping, muscular dystrophy, and severe myotome defects. Postoperative kidney failure occurred more frequently in patients with TMEM260-associated truncus arteriosus than in those with truncus arteriosus of other etiology (30% vs. 5%, p=0.005). Notably, kidney failure developed only after procedures involving severe hypothermia (two occasions out of the four cardiac surgeries performed in the three children), including in a patient without pre-existing kidney involvement.

Conclusions: *TMEM260*-associated kidney involvement is incompletely penetrant and modulated by temperature. TMEM260-dependent mannosylation may confer protection against heat stress–induced renal injury.

Podocin oligomers regulate the ordering of nephrin chains

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Keywords: nephrin, podocin, slit diaphragm, pore size, *NPHS2*, nephrotic syndrome

NPHS2 is the most frequent causative gene in podocytopathies. The encoded podocin homooligomerizes through its C-terminal and binds nephrin in the slit diaphragm. The *NPHS2* R229Q variant is only pathogenic when trans-associated to specific 3' variants causing R229Q podocin to form distorted heterooligomers. Based on structural models of podocin oligomers and electron microscopy data of the slit diaphragm, we hypothesized that podocin oligomers regulate the distance between nephrin molecules.

To examine the effect of podocin on the distance between nephrin molecules, nephrin constructs labelled with YPet or mCherry/mRuby were transitionally coexpressed in HEK-293 cells. We assessed the nephrin-nephrin distance based on the FRET efficiency between YPet and mCherry/mRuby in living cells.

Podocin markedly increased the FRET efficiency between nephrin molecules, reflecting reduced and ordered nephrin-nephrin distances. Its effect was abolished by pathogenic podocin variants. Pathogenic R229Q heterooligomers exhibited increased FRET efficiency between their PHB domains that correlated with a larger nephrin-nephrin distance, thereby explaining the associated mild phenotype. The effect of pathogenic and benign R229Q heterooligomers separated sharply in their effect on nephrin-nephrin spacing (P=1.19E-33). Based on an intermediate effect on the nephrin-nephrin distance and five families with late-onset FSGS, we reconsider the R229Q-R286Tfs*17 association as pathogenic, but via a mechanism different from other R229Q associations and with incomplete penetrance.

In conclusion, podocin provides regularly spaced intracellular anchor points for nephrin chains. Podocin homo-oligomerization affects the nephrin-nephrin spacing, thereby providing an explanation for albuminuria in patients with podocin dysfunction and for the interallelic interactions of R229Q.

Src Family Kinases Hck, Fgr, and Lyn Drive Disease Progression in Experimental Immune Complex–Mediated Glomerulonephritis

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Keywords: glomerulonephritis, cell signaling, Src kinases

In immune complex–mediated glomerulonephritis, circulating immune complexes are deposited along the glomerular basement membrane, leading to local inflammation and progressive deterioration of renal function. The signaling pathways involved in disease pathogenesis remain poorly understood.

Our aim was to investigate the role of Src family tyrosine kinases expressed in myeloid cells (Hck, Fgr, and Lyn) in a model of immune complex–mediated glomerulonephritis, with particular emphasis on the individual contribution of each kinase.

Immune complex–mediated glomerulonephritis was induced using an accelerated nephrotoxic nephritis model. Animals were preimmunized with sheep IgG, followed by induction of glomerulonephritis using sheep serum raised against mouse glomeruli (nephrotoxic serum, NTS). Urine samples were collected every second day to monitor renal function, and blood and tissue samples were obtained at the end of the experiment. Renal function was assessed by measuring urinary albumin levels as well as urinary and plasma creatinine concentrations. Structural kidney damage and immune cell infiltration were analyzed by histological methods (PAS staining, immunohistochemistry), while the composition and activation status of renal immune cell populations were quantified by flow cytometry.

NTS treatment induced severe glomerulonephritis in wild-type mice, characterized by marked proteinuria, elevated serum creatinine levels, crescentic glomerular lesions, glomerulosclerosis, and significant leukocyte infiltration. In line with our previous findings, $Hck^{-/-}Fgr^{-/-}Lyn^{-/-}$ triple knockout mice were completely protected from disease development. $Hck^{-/-}$ single knockout mice also exhibited significant protection, whereas $Fgr^{-/-}$ and $Lyn^{-/-}$ single knockout mice developed severe glomerulonephritis comparable to that observed in wild-type animals.

Src family tyrosine kinases expressed in myeloid cells, particularly Hck, play a critical role in the development and progression of immune complex–mediated glomerulonephritis. The kinases investigated in this study represent promising therapeutic targets for the treatment of immune complex–mediated glomerulonephritides.

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Renal DNase I expression is associated with disease activity in human lupus nephritis

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Keywords: nephrology, systemic lupus erythematosus, lupus nephritis, DNase1

Background: Lupus nephritis (LN) occurs in half of the patients with systemic lupus erythematosus. The accumulation of autoantibodies against nuclear and cellular components leads to immune complex formation and tissue damage. Endonuclease enzymes, including deoxyribonuclease 1 (DNase1), may help clear the nuclear debris, but their exact role in LN is still unknown. The aim of this study is to define the expression pattern of DNase1 and correlate to disease activity in LN.

Methods: We examined renal specimens and clinical data of patients who underwent kidney biopsy in our centre between 2005 and 2020. DNase1 expression was assessed by immunofluorescence in paraffin-embedded samples. ImageJ and SPSS Statistics software were used to determine the immunofluorescent intensity and analyse the data, respectively.

Results: From the 91 kidney biopsies, DNase1 staining was performed on 82 samples based on availability, and 71 were analysed after quality control. Median age was 36 (min. 18, max. 74) years, 86% were female. Laboratory measurements were available in 68 cases. Glomerular intensity of DNase1 showed positive correlation with serum creatinine (r_s=0.311; p=0.007), urea (r_s=0.249; p=0.041), potassium (r_s=0.285; p=0.018), and time to remission (r_s=0.353; p=0.018); while a negative correlation with eGFR (r_s= -0.295; p=0.015) was observed. Tubular DNase1 intensity showed correlation solely with glomerular intensity.

Conclusion: Glomerular DNase1 expression correlates with the severity of LN and the response to treatment. We theorize that elevated DNase1 expression may serve as a compensatory response to glomerular damage caused by immunocomplexes. Glomerular DNase1 intensity may serve as a potential marker of disease progression.

Session IV.
Revisiting Physiology Education / Élettan oktatás

Revisiting Physiology Education: Current Challenges and Structured Dialogue for Future Directions

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Keywords: physiology education, challenges, educational methods, assesment

Physiology education is currently challenged by a combination of structural, pedagogical, and cultural changes that increasingly question the effectiveness of traditional teaching approaches. Declining lecture attendance, expanding student cohorts, and growing concerns regarding academic integrity suggest that established educational models may require critical reassessment. At the same time, difficulties in recruiting and retaining qualified teaching staff raise concerns about the long-term sustainability of physiology education.

The aim of this session is to provide a structured forum for identifying and critically discussing key challenges in physiology education, while also exploring potential directions for improvement. The session will start with concise and focused presentations of key problems, as seen by the panel, and is followed by moderated discussion and knowledge exchange, thus the majority of the session is dedicated to discussion and participant engagement.

The session will address several interrelated themes, including the evolving role and relevance of traditional lectures, challenges in academic workforce development, and emerging issues related to examination-related misconduct. The adequacy of current assessment strategies will be considered, particularly in relation to competency-based education. In addition, the structure and pedagogical value of practical physiology teaching will be examined. The impact of increasing student numbers and deteriorating teacher-to-student ratios, leading to mass education formats, will also be discussed.

Participants will be encouraged to share institutional experiences, challenge assumptions, and contribute additional perspectives. The aim is to identify common patterns, highlight promising practices, and foster collaborative approaches to improving physiology education.

The expected outcome is a set of shared insights and actionable considerations that may inform curriculum development, teaching innovation, and institutional decision-making in physiology education.

Session V.
Circadian Rhythms from Research to Clinical Practice

Adipose tissues on a schedule: the impact of time-restricted feeding on rhythmicity, immune cell trafficking, and inflammation

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Keywords: Time-restricted feeding, adipose tissue, leukocyte, mouse model, inflammation

Feeding time is a major regulator of circadian organization in metabolic tissues. Disruption of feeding patterns desynchronizes central and peripheral clocks and contributes to metabolic inflammation. Adipose tissue plays a key role in this process through coordinated regulation of adipokine secretion, immune cell composition, and gene expression. Time-restricted feeding (TRF) has emerged as a potential intervention to improve metabolic and inflammatory homeostasis, but its tissue-specific molecular effects remain incompletely understood.

We aimed to investigate the effects of TRF on adipose tissue inflammation by integrating gene expression changes with alterations in immune cell composition and trafficking-related processes.

Male wild-type C57BL/6 mice were assigned to four groups: standard chow or high-fat (HF) diet *ad libitum*, or TRF with either diet restricted to the first 10 hours of the active phase. After four weeks, epididymal and subcutaneous white adipose tissues, brown adipose tissues, and blood samples were collected. Gene expression of inflammatory cytokines, adipokines, and clock genes was analyzed alongside leukocyte populations and adhesion-related markers.

TRF reduced the expression of proinflammatory cytokines and adipokines in WAT under both dietary conditions. HF feeding increased inflammatory gene expression and disrupted circadian patterns, whereas TRF restored rhythmic regulation. These molecular changes were accompanied by reduced immune cell infiltration, characterized by decreased neutrophil abundance and relative enrichment of regulatory T cells. In parallel, TRF influenced leukocyte trafficking dynamics, reflected by changes in adhesion molecule expression and temporal distribution of circulating immune cells.

Our findings highlight coordinated circadian and immunometabolic mechanisms underlying the beneficial effects of TRF.

Time-restricted feeding alleviates contact dermatitis by modulating leptin levels

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Keywords: allergy, diurnal rhythm, contact dermatitis, cytokines, neutrophils

In modern societies, irregular and unhealthy eating behavior is increasingly prevalent, contributing to the development of both metabolic and inflammatory disorders. Limiting food access to a specific time window of the day, even without caloric restriction, helps prevent and treat metabolic diseases. Given the close connection between metabolism and immune function, we examined how meal composition and meal timing influence contact hypersensitivity (CHS), which is a mouse model of human allergic dermatitis—a disease that affects circa 20% of the population. Under conditions with ad libitum (AL) food access, high-fat (HF) diet resulted in impaired metabolic state and a more severe form of inflammation. Increased ear thickness, leukocyte infiltration, pustule formation, IL-1β, CXCL2 levels and markedly delayed resolution of the inflammation were observed in HF-AL animals. Time-restricted feeding (TRF) reduced HF diet-induced weight gain and exacerbation of the inflammatory symptoms, even when started after disease onset. In HF-AL mice, serum leptin levels were elevated and displayed a phase-shifted diurnal pattern compared to the normal diet (NC) group, whereas TRF reversed these effects. NC-fed *db/db* mice, which lack the longer form of the leptin receptor and produce high levels of leptin, exhibited worsened CHS. Blocking the leptin receptors alleviated the inflammatory symptoms in both *db/db* and HF-AL wild type mice. Our results suggest that leptin may contribute to pustule formation in non-infectious dermatitis in humans; hence, both TRF and local inhibition of leptin receptors can individually and in combination serve as effective new tools in managing allergic contact dermatitis.

Circadian rythm and cancer

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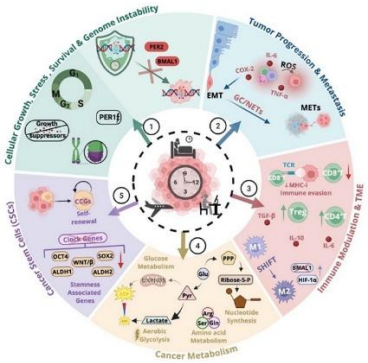
Keywords: cancer evolution/progression, circadian rythm/genes, oncotherapy

Nowadays medical literature is increasingly dealing with the interactions between tumor evolution, the effectiveness of oncotherapies and the circadian rhythm. Obviously, the fact that most tumors arise in our body over years/decades and that the effect of different cancer treatments develop over weeks/months somewhat contradicts this theory and the direct connection. Nevertheless, both our daily rhythm of life and the circadian functioning of our cells can have a proven influence on the development of cancer at countless points. In this summary, we will try to briefly discuss these key points based on medical literature.

Potential factors involved in tumor evolution: 1. Our daily rhythm, eating habits, and gut microbiome. 2. Healthy lifestyle, regular exercise vs. weight gain, and secondary metabolic diseases. 3. Sleep disorders, depression, secondary harmful habits and cancer risk. 4. Shift work and the risk of chronic diseases, like cancer. 5. The role of melatonin in cell regulation.

Factors that may be potential role in tumor progression: 6. Biological clock and cell function regulation. 7. Relationship between circadian genes and tumor growth/metabolism. 8. The “epigenetic clock”. 9. The role of hypoxia-inducing factors. 10. Tumor progression and secondary circadian/hormonal consequences. 11. Circadian effects on microenvironment and immune system function. 12. Diurnal differences in tumor symptomatology.



Circadian clock regulation in cancer hallmarks. (Robinson, Journal Of Biological Rhythms, 2026)

Factors influencing the effectiveness of oncotherapies: 13. The role of cancer care in daily rhythm and lifestyle. 14. Is there any influence on treatment effects at different periods of the day? 15. The effect of multi-day and multi-week treatments on the responses of normal and tumor tissues. 16. The effect of metronomic chemotherapy and chrono-immunotherapy.

Summary: It is difficult to absolutely separate our lives and our diseases, and from cellular work to the highest human functions, the events of a single day sum up into weeks, months, years, decades. Thus, it is understandable that “ones of our days” can ultimately turn into a serious illness, but “several disparate days” can even facilitate the path to recovery.

Circadian rhythms and emergency care

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Introduction: To evaluate emergency patient care from a chronophysiological perspective, examining circadian variations in symptom presentations and physiological parameters in single-centre retrospective analysis at the Department of Emergency Medicine, Clinical Centre, University of Pécs, Hungary (August 2022 to August 2023).

Materials and methods: Patient data including symptom categories, time of presentation (divided into eight 3-hour intervals), sex and age were collected. A total of 32 977 patient records were analysed. Symptoms were classified using the International Classification of Diseases, 10th Revision. For hypertension, random proportionally stratified sampling was performed (n=120). Primary and secondary outcome measures: Primary outcomes were the circadian distribution of emergency presentations across 14 symptom categories and their variation by time of day, age and sex. Secondary outcomes included diurnal variation in hypertension-related cases, examining blood pressure, pulse rate, triage time and medication use.

Results: Cardiovascular cases peaked between 09:00 and 12:00 ($p<0.001$), toxicological emergencies between 18:00 and 21:00 $p<0.001$) and endocrine–metabolic cases between 12:00 and 15:00 ($p<0.001$). In hypertensive patients, the lowest systolic pressure occurred between 12:00 and 15:00 ($p=0.037$). More patients presented on weekdays than weekends ($p=0.013$).

Conclusions: Symptom presentations in emergency care follow distinct circadian patterns, highlighting the influence of biological rhythms on clinical demand. Recognition of these temporal trends may support more effective ED scheduling and resource allocation.

Heart rate patterns in nocturnal sleep: identifying biomarkers of human circadian rhythmicity

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Keywords: circadian rhythm, peripheral clocks, heart rate, sleep

Background: A recent critical appraisal of the field of circadian medicine reports that, despite the surrounding enthusiasm, there is a noticeable lack of effective implementation and a striking stagnation in the development of chronomedicine. The authors identify the absence of reliable biomarkers and normative measurements that can be obtained using wearable devices as one of the reasons for this deadlock.

Aims: We test the hypothesis that the phase and relative amplitude of the nocturnal minima in heart rate (HR) reflect the phase and amplitude of the circadian modulation of sleep, respectively.

Methods: HR was continuously assessed in human volunteers for 7 days (N = 48; Cortrium C3+), along with core body temperature (Calera Research), wrist actigraphy (GeneActive), and sleep–wake behavior (sleep logs). Subjective indices of chronotype were evaluated using standard questionnaires. In addition, electrocardiogram (ECG) recordings derived from nocturnal polysomnography were analyzed in N = 251 subjects. HR during nocturnal sleep was processed using outlier exclusion, block median smoothing, and low-pass Fourier transform filtering (removing harmonics with wavelengths < 120 minutes).

Results: The findings indicate that the nadir in HR occurs around 4:00 AM under both home and laboratory conditions, with 57% of the latter cases coinciding with stage N2 sleep. Age-associated attenuation of HR decline and a phase advance of the nadir were revealed. The bathyphase of HR reflected both subjective (questionnaire-based) and objective (actigraphy-based) indices of chronotype. Sleep onset time was only a marginal predictor of HR bathyphase.

Conclusion: The typical nocturnal nadir in HR occurs during late, superficial sleep rather than during early, deep sleep. This nadir may serve as a putative indicator of circadian amplitude, phase, and chronotype. Previously reported late-night minima in circulating adrenaline may represent a potential physiological basis for these findings.

Associations between chronotype, diurnal cortisol rhythm and insomnia severity among healthcare professionals in shift work

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Keywords: chronotype; shift work; insomnia; cortisol

Background: Chronotype is a primary determinant of tolerance to shift work; however, its physiological correlates remain insufficiently explored in healthcare professionals. This study aimed to investigate the associations between chronotype, perceived stress, sleep quality, and health behaviours in shift-working nurses and physicians.

Methods: A cross-sectional study was conducted with 451 participants (77% female; mean age 42 ± 11 years). Instruments included the Perceived Stress Scale (PSS), Athens Insomnia Scale (AIS), Patient Health Questionnaire Somatic Symptom Scale (PHQ-15), Sleep Hygiene Index (SHI), and the reduced Morningness–Eveningness Questionnaire (rMEQ). Additionally, salivary cortisol, blood pressure, and pulse were measured in a physiological subsample (n = 40).

Results: Evening chronotypes exhibited higher insomnia prevalence (70%) and significantly elevated AIS scores (8.2 ± 4.2, $p < 0.001$). The general linear model ($F = 63.75$, $p < 0.001$) explained 45.9% of the variance in insomnia severity, with somatic symptoms ($B = 0.313$), sleep hygiene ($B = 0.112$), perceived stress ($B = 0.081$), and eveningness ($B = -0.092$) emerging as significant predictors ($p < 0.05$). Older age and higher night shift frequency also contributed to poorer sleep. The physiological subsample revealed poorer regulatory outcomes for evening types, who demonstrated a significantly attenuated cortisol awakening response ($p = 0.02$) and a flatter diurnal cortisol slope during day shifts ($p = 0.01$) compared to other chronotypes.

Conclusions: Evening chronotype is linked to impaired sleep quality and altered HPA-axis regulation. These findings highlight the necessity of chronotype-based scheduling and targeted behavioral interventions to mitigate circadian misalignment and improve occupational health outcomes in healthcare settings.

Session VI. Mechanisms of brain disorders: cellular, vascular and stress-responsive pathways

Modelling the aging-Parkinson axis using patient-derived cellular systems

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Aging is the primary risk factor for most chronic and neurodegenerative diseases, yet the molecular and cellular mechanisms underlying human brain aging remain poorly understood. Current knowledge is largely derived from animal models, despite well-established differences between species in cellular lifespan, morphology, and molecular profiles. Human neurons, astrocytes, and microglia exhibit distinct transcriptomic, epigenetic, and functional properties, highlighting the need for human-specific models to accurately capture aging-related processes.

A major barrier to studying human brain aging is the limited access to living neural tissue. To overcome this challenge, our work leverages patient-derived cellular reprogramming approaches to generate human brain cells in vitro. In particular, direct reprogramming of somatic cells into neural lineages preserves age-associated features of the donor, enabling the study of cells across the human lifespan. This contrasts with induced pluripotent stem cell-based approaches, which reset cellular age and yield rejuvenated cells. Together, these complementary systems provide a powerful platform to investigate the biology of human brain aging.

Using these state-of-the-art models, our research program aims to define the molecular and functional signatures of aging in neurons, microglia, and astrocytes. By identifying conserved and cell type-specific aging pathways, this work seeks to uncover key drivers of age-related cellular decline and vulnerability. Ultimately, these insights will inform the development of targeted anti-aging interventions and improve the integration of aging biology into regenerative and precision medicine strategies.

Direct Neural Reprogramming as a Cellular Model of Human Brain Aging and Neurodegeneration

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Direct reprogramming of human fibroblasts into induced neurons (iNs) and induced astrocytes (iAs) provides a powerful model to study human brain aging and age-related neurodegenerative diseases. This method bypasses pluripotency, therefore iNs and iAs retain the donor's genetic, epigenetic, and biological aging signatures, making them uniquely suitable for investigating age-dependent cellular pathways and patient-specific disease mechanisms.

In our laboratory, this platform is integrated with automated high-content imaging, quantitative morphology, and multi-omics analyses to characterise aging and autophagy in human neurons and astrocytes. These approaches have revealed marked cell-type-specific differences in autophagy regulation, highlighting distinct neuronal and glial vulnerabilities. Through transcriptomic, proteomic, phosphoproteomic, DNA-methylation-based aging clocks, we aim to identify molecular networks that drive neuronal decline and define potential rejuvenation targets.

Beyond fundamental research, directly reprogrammed iNs enable reverse translational approaches in which patient-derived neurons are used to investigate clinically applied or candidate therapeutics. Studies performed in the context of felodipine and cariprazine treatment have shown donor-dependent cellular responses, linking autophagy, neuronal complexity and electrophysiological properties to clinical variation. These findings underscore the potential of iNs for precision medicine and for identifying patient subgroups most likely to benefit from specific interventions.

Building on these efforts, our MiND+ framework aims to establish a human neuron-based platform for drug discovery, target validation and patient stratification in cognitive and neurodegenerative disorders. Together, these approaches demonstrate how direct cellular reprogramming can bridge fundamental aging biology with translational applications and support the development of future therapeutic strategies.

The role of pericytes in age-related brain disorders

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Keywords: aging, brain pericyte, neuroinflammation, microinfarcts

Cerebral pericytes are mural cells that envelop brain microvessels and are structurally classified into ensheathing, mesh, and thin-strand subtypes. These cells undergo functional and molecular alterations during ageing, which may be further exacerbated in age-related pathologies.

Microinfarcts are often associated with aging. In our study, microinfarcts were modeled by injecting 10 μm microspheres into the circulation of mice, leading to capillary occlusion. This obstruction increased α-smooth muscle actin (α-SMA) expression in pericytes in both young and aged animals. However, baseline levels of α-SMA, its encoding gene Acta2, and its regulator TGF-β1/Tgfb1 were reduced in aging cerebral microvessels, indicating an age-dependent decline in contractile capacity.

Given that aging is commonly associated with a pro-inflammatory phenotype, we further investigated the effects of tumor necrosis factor-α (TNF-α) on pericyte function. Intracerebral administration of TNF-α induced pericyte contraction and consequent capillary constriction, leading to reduced red blood cell (RBC) flux downstream. In contrast, peripherally derived TNF-α caused dilation of brain microvessels and penetrating arterioles despite concurrent pericyte contraction, resulting in increased downstream RBC flux.

These findings highlight the complex and context-dependent role of pericytes in regulating cerebral microcirculation and underscore their critical involvement in age-related microvascular dysfunction.

Transient receptor potential ankyrin 1 ion channels may modulate the stress responses through glia-mediated mechanisms in the mouse model of posttraumatic stress disorder

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Keywords: PTSD, TRPA1, fear extinction, astrocyte, microglia

Introduction: Posttraumatic stress disorder (PTSD) is a mental illness resulting from disturbed stress adaptation. Transient receptor potential ankyrin 1 (TRPA1) is a non-selective cation channel expressed on several stress-related brain areas. Our recent study using the electric foot shock protocol of PTSD confirmed *Trpa1* mRNA downregulation in a stress sensitive brain hub and revealed aggravated fear responses in *Trpa1* knockout (KO) mice, suggesting the modulatory impact of TRPA1 on PTSD symptomology. As TRPA1 is expressed on the glial cells of the brain, we hypothesize that the ion channel may regulate fear extinction through glia-mediated mechanisms.

Methods: The electric foot-shock model of PTSD was performed on two sets of male *Trpa1* wild-type (WT) and KO mice. During the fear conditioning phase, animals were exposed to foot shocks combined with acoustic stimuli, followed by a four-week resting period. Then conditioned fear tests (CFT) were carried out using situational reminders without foot shock. In the first subset of mice, we examined the extinction of fear response (freezing reaction) followed by serum corticosterone (CORT) measurements. In the other group, we evaluated the jumping response indicating psychomotor arousal and determined the neuroinflammation using glia specific immunomarkers in PTSD-related brain regions (hippocampus, central nucleus of amygdala, medial prefrontal cortex - mPFC).

Results: Stress increased freezing in both genotypes, but significantly higher jumping rates were restricted to KOs. Upon repeated introduction to the trauma context/cues, the fear response was extinguished; however, this process was slower in KOs than WTs. Additionally, relapse occurred in KOs only, accompanied by reduced CORT levels. Foot shock activated astrocytes in WTs (except mPFC) without influencing the microglia response, while KO mice reacted to stress with increased microglia, but not astrocyte, activation across all regions.

Conclusions: TRPA1 channel may promote fear extinction and prevent the relapse of fearful memories through regulating stress-induced CORT responses and neuroinflammatory processes.

Session VII.
Novel mechanisms of cerebral blood flow regulation: from molecules to humans

Molecular bases of the opposite flow-induced responses of pre- and post-circle of Willis arteries

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Keywords: arachidonic acid metabolism; cerebral blood flow regulation; mechano-transduction; nitric oxide signaling; small cerebral arteries.

Introduction: Increases in flow elicit dilations in the basilar artery (BA, pre-Willis, preWa) supplied by the posterior cerebral circulation and ensuring efficient blood supply to the circle of Willis. In contrast, increases in flow elicit constrictions in the post-circle of Willis (postWa), middle cerebral artery (MCA). Mediators of flow-dependent responses include arachidonic acid (AA) metabolites and nitric oxide (NO). We hypothesized that molecular basis of flow-dependent responses is differentially represented in these cerebral arteries.

Methods: The expressions of key enzymes of the AA pathway-cyclooxygenases (COX1/COX2), cytochrome P450 hydroxylases (Cyp450), thromboxane synthase (TXAS), thromboxane A2 (TP) receptor, prostacyclin synthase (PGIS), prostacyclin (IP) receptor (IP); neuronal nitric oxide synthase (nNOS), and endothelial nitric oxide synthase (eNOS)-in the BA and MCA from rats (n = 20) were determined by western blotting. Transcriptome analysis in preWa and postWa from rats (n = 25) was assessed by RNA sequencing.

Results_ In preWa compared to postWa, COX1/2 and Cyp450 protein expressions were lower, PGIS was higher, TXAS and nNOS/eNOS were similar, TP receptors were lower, and IP receptors were higher. Gene expressions of vasodilator canonical pathways were higher in preWa, whereas vasoconstriction canonical pathways were higher in postWa.

Conclusions: Molecular basis and mediators of flow-dependent vasomotor signaling are differentially expressed in the pre- and post-circle of Willis cerebral arteries, corresponding to their opposite flow dependent vasomotor function.

The role of arachidonic acid metabolites in the regulation of cerebral blood flow

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Cerebral blood flow (CBF) regulation is critically dependent on endothelial function and the balance of vasoactive mediators, among which arachidonic acid (AA) metabolites play a central role. Evidence from experimental and clinical studies indicates that high salt intake, oxidative stress, and alterations in the renin–angiotensin system profoundly modulate these pathways. Under physiological conditions, flow-induced dilation (FID) in cerebral arteries is mediated predominantly by nitric oxide (NO) and vasodilatory AA metabolites, including prostacyclin and epoxyeicosatrienoic acids (EETs). However, in conditions such as high salt intake, there is a shift in the mediators of FID toward vasoconstrictor AA derivatives, particularly cyclooxygenase (COX)-derived prostanoids and cytochrome P450 metabolites like 20-hydroxyeicosatetraenoic acid (20-HETE).

Experimental studies in Sprague-Dawley and Dahl salt-sensitive rats demonstrate that high salt diets impair endothelium-dependent dilation in middle cerebral arteries, largely through increased production of reactive oxygen species (ROS) and reduced NO bioavailability. This is accompanied by an increased contribution of COX-1-derived vasoconstrictor prostanoids and 20-HETE, which promote vascular dysfunction and elevate cerebrovascular resistance. Clinical data further support the role of COX-1 activity in salt-induced microvascular dysfunction in humans. Importantly, modulation of the renin–angiotensin system significantly influences these mechanisms. Reduced angiotensin II (Ang II) levels under high salt conditions exacerbate endothelial dysfunction, while low-dose Ang II supplementation restores FID by rebalancing NO and AA metabolite signaling and reducing oxidative stress.

At the molecular level, oxidative stress alters AA metabolism by enhancing the production of vasoconstrictor metabolites and suppressing vasodilatory pathways. Interactions between Ang II signaling, NADPH oxidase-derived superoxide, and endothelial calcium dynamics further regulate the synthesis of AA metabolites. Additionally, AT1 receptor activity has been shown to modulate cerebral vasomotor responses through redox-sensitive mechanisms involving both COX and cytochrome P450 pathways.

Overall, these findings highlight that dysregulation of AA metabolites—particularly the shift toward COX-derived vasoconstrictors and 20-HETE—represents a key mechanism underlying impaired CBF autoregulation in conditions of high salt intake and oxidative stress. Targeting these pathways may provide therapeutic strategies for preserving cerebrovascular function and preventing stroke-related complications.

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The significance and mechanism of age-related neurovascular uncoupling: the role of age-dependent IGF-1 deficiency

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Neurovascular coupling (NVC) enables activated brain regions to precisely meet their energy demands. With aging, NVC weakens, which may contribute to cognitive decline. One of the most important age-related neuroendocrine changes is decline in circulating IGF-1 level, however, its effect on NVC decoupling has not been established.

We show that in conditional knockout of liver specific production of IGF-1 causes endothelial dysfunction and impaires glutamate-mediated, neuronal activation-induced increase in CBF via altering the production of astrocytic gliotransmitters. These changes are associated with cognitive decline. We also show in humans that age-related IGF-1 deficiency correlates with reduced neurovascular hyperemia.

Preserving the IGF-1 signaling pathway and supporting endothelial function may be crucial for preserving cognitive function in older age. Therapeutic approaches may potentially offer reversible effects on aging-related neurovascular dysfunction.

Changes in vasomotor responses of extra- and intracranial arteries and placental vascular structure in aged pregnant rats

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Keywords: middle cerebral artery, carotid artery, placenta, pregnancy, aging

Introduction/Aims: Cerebral blood flow is regulated by complex mechanisms via changes in the resistance of extra- and intracranial arteries. These mechanisms are - in part - activated by changes in intraluminal pressure and vasoactive molecules, which may be affected by aging and pregnancy. We hypothesized that age-dependent changes in the vasomotor function of extra- and intracranial arteries and changes in the vascular structure of placenta in young and aged pregnant rats might predict the development of preeclampsia.

Methods: Young and aged (3-4 months and 8-10 months old, n=36) pregnant (YP and AP) and nonpregnant (YNP and ANP) female rats were anaesthetized, and their arterial blood pressure (BP) and heart rate (HR) were measured. Common carotid artery (CCA) and middle cerebral artery (MCA) were isolated, and their vasomotor responses were measured with wire myography or pressure angiography respectively. Contractile responses of CCA were obtained in response to phenylephrine (Phe), angiotensin II (AngII), and U46619 (thromboxane A2 analogue), and endothelium-dependent relaxations were measured to acetylcholine (Ach). Specific inhibitor of nitric oxide synthase (L-NAME) and inhibitor of prostanoid production by cyclooxygenase (indomethacin) were also used. In MCA pressure-diameter responses and vasomotor reactivity were obtained. By using hematoxylin-eosin staining the fetal and maternal vascular structure of the placenta was also visualized.

Results: In YP and ANP rats BP decreased compared to YNP (YNP:116/98±15/12; YP:98/65±8/8; ANP:89/65±4/6 mmHg; $p<0,05$). In pregnant rats, the contractile responses of CCA were higher than in nonpregnant rats. AngII-induced contractions decreased in AP compared to ANP. Endothelium-dependent relaxation was enhanced in the presence of indomethacin in AP compared to ANP. In MCA both pressure-induced diameter changes and contractile response to U46619 were reduced in AP rats. The fetal capillary density in the placenta decreased slightly in AP, while the proportion of maternal blood vessels remained unchanged.

Conclusion: These findings by showing substantial changes in the contractile responses of extra- and intracranial arteries and vascular structure of the placenta in aged pregnancy compared to young pregnancy, suggest that these changes may contribute to the attenuation of cerebral blood flow regulation and placental circulation, both of which may increase the risk of preeclampsia development.

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Regional characteristics of glymphatic dysfunction in aneurysmal subarachnoid hemorrhage

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Objective: Subarachnoid hemorrhage (SAH) leads to glymphatic dysfunction via obstruction of periarterial CSF spaces, precluding sufficient interstitial fluid flow and clearance demonstrated by MRI after intrathecal administration of contrast agent. We sought to investigate alterations of glymphatic flow in SAH with non-contrast MRI methods and establish their association with CSF/serum biomarkers and clinical outcomes.

Methods: 24 consecutive patients (17 females, 57,4±12,9 years) with aneurysmal SAH were enrolled prospectively. Imaging protocol consisting of multishell DWI (b-values 0, 1000, and 2000 s/mm^2), T2-space, MPRAGE and TOF angiography was acquired on a 3T MRI scanner within 24 hours of the ictus and on the 7th postictal day. Prior to each MRI scan, CSF and blood samples were collected to measure levels of AQP-4, neurofilament light-chain and amyloid-beta-42. A control group of 16 age and sex matched healthy volunteers underwent the same imaging protocol and blood sample collection. Segmentation and volumetry of perivascular spaces (PVS) on T2space were used as a proxy for impairment of periarterial influx. Extracellular free water fraction (FWF) was calculated from diffusion kurtosis (DKI) metrics. DTI-ALPS index was employed to quantify disturbance of perivenous outflow. To account for microstructural white matter changes, Tract-Based Spatial Statistics (TBSS) were performed on DTI data using the diffusion and kurtosis tensor previously reconstructed in DIPY.

Results: Compared to control subjects, PVS volume of the SAH group decreased significantly in the midbrain ($p<0.001$) and basal ganglia (BG) ($p=0.017$) and expanded ($p=0.004$) in the centrum semiovale (CSO). The volume of BG PVS on the 1st day correlated significantly with CSF-AQP-4 levels on the 7th day ($R=-0.583$, $p=0.011$) and the occurrence of vasospasm ($R=-0.629$, $p=0.016$). FWF significantly increased in the CSO and BG ($p<0.001$ and $p=0.027$, respectively) in SAH patients as compared to healthy controls. FWF-CSO showed correlation with GOSE at 3 months ($R=-0.571$, $p=0.026$). DTI-ALPS index in the SAH group significantly decreased ($p<0.012$) on the 1st day, further decrease at day 7 was not significant ($p=0.072$), while PSMD index showed more pronounced group difference ($p<0.001$). TBSS evaluating the FA/MD/RD/AD changes found association between clinical outcome at 3 months (measured by mRS) and RD change in the forceps major, as well as NfL levels and AD change in the superior longitudinal fasciculus (corrected $p<0.05$).

Conclusion: Subarachnoid hemorrhage induced glymphatic dysfunction is quantifiable by non-contrast MRI metrics, which are associated with CSF biomarkers, and clinical outcomes, supporting the role of glymphatic alterations in secondary brain injury and recovery in SAH patients.

Association of modifiable risk factors with retinal vascular health and cognitive performance in an occupational cohort (Semmelweis Study)

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Keywords: cognitive performance, retinal blood vessel, vascular cognitive impairment and dementia

Introduction: Hungary faces one of the highest burdens of unhealthy aging in Europe, with age-related cognitive impairment representing a major public health challenge. Microvascular dysfunction is an early driver of cognitive decline, and detecting these alterations before the onset of vascular cognitive impairment and dementia (VCID) is critical for prevention. The Semmelweis Study, an occupational cohort study, enables identification of preclinical vascular–cognitive changes in working-age adults. Retinal and cerebral microvessels share structural and functional similarities, making retinal imaging a non-invasive proxy for brain microvascular health. We tested the hypothesis that lower retinal arteriovenous ratio (AVR) is associated with impairments in cognitive performance and presence of modifiable lifestyle factors.

Methods: We analyzed 405 participants with retinal imaging and Cambridge Neuropsychological Test Automated Battery (CANTAB) assessments. AVR was derived from non-mydriatic fundus images. Mediterranean diet adherence and physical activity were assessed using validated questionnaires. Participants were categorized into AVR tertiles, and differences between low and high tertiles were evaluated.

Results: Participants in the lowest AVR tertile had lower Rapid Visual Information Processing Accuracy (90±10% vs. 92±9%, $p<0.01$) and poorer Spatial Working Memory Strategy scores (8.54±2.67 vs. 7.28±2.69, $p<0.01$). They also showed lower Mediterranean diet adherence (7.34±2.29 vs. 7.89±2.17, $p<0.05$) and higher mean arterial pressure (92.76±12.52 vs. 83.19±15.33, $p<0.01$).

Conclusion: Lower AVR was associated with subtle reductions in cognitive performance, supporting microvascular dysfunction in the central nervous system as an early pathway linking cardiovascular risk to cognitive decline. Associations with blood pressure and diet highlight modifiable targets for early prevention of VCID.

Session VIII.
Cardiac electrophysiology

Investigation of cardiac repolarization reserve: the importance of ventricular Ikur inhibition

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Background: The ultrarapid delayed rectifier potassium current (IKur) has long been considered an atrial-specific current with no functional role in the ventricles, despite evidence of its expression in ventricular myocytes.

Objective: This study aims to investigated the potential role of IKur in ventricular repolarization.

Method**:** Experiments were performed on cardiac preparations obtained from Beagle dogs of both sexes. Kv1.5 protein expression was determined by immunocytochemistry in canine and undiseased human cardiac tissue samples. Action potentials were recorded using conventional microelectrode techniques in canine and human cardiac preparations, and membrane ionic currents were measured using the whole-cell onfiguration of the patch-clamp technique in isolated cardiomyocytes.

Result: Kv1.5 protein expression was detected in canine atrial, ventricular, and Purkinje fibre cells, as well as in undiseased human left ventricular myocardium. In canine atrial preparations, IKur inhibition shifted the plateau phase of the action potential into the positive voltage direction, and consequently shortened action potential duration. Application of 100 μM 4-AP revealed ionic currents of similar magnitude in ventricular myocytes and Purkinje fibres, which were attributed to IKur. Inhibition of IKur caused prolongation of the action potentials recorded from endocardial and midmyocardial preparations, as well as from Purkinje fibres. Under conditions of attenuated repolarization reserve, IKur inhibition induced augmented repolarization lengthening and frequent early afterdepolarizations (EADs).

Conclusion: we suggest that IKur which previously was considered an atrial-specific current exists and plays a significant fine tuning role in both canine and human ventricles. Also, IKur may importantly contribute to ventricular repolarization reserve, although its precise role remains to be investigated.

Assessment of intracellular calcium handling and cardiac alternans in a canine model of elite exercise

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Keywords: cardiac alternans, calcium handling, athlete's heart, cardiac arrhythmias

Regular physical training is widely recognized for its beneficial effects on the cardiovascular system and overall quality of life. However, increasing number of evidence indicates a higher prevalence of cardiac arrhythmias and an increased incidence of sudden cardiac death (SCD) among elite athletes. The underlying mechanisms of these exercise-induced adverse outcomes remain incompletely understood. Here we report on a large animal model of athlete's heart and demonstrate that intensive exercise-induced electrical remodelling facilitates the development of cardiac alternans, which may serve as a substrate for arrhythmogenesis.

A total of 24 Beagle dogs of both sexes were randomly assigned to sedentary control or trained groups. The trained group was subjected to a 16-week vigorous treadmill running protocol. Following the training period, comprehensive electrophysiological and molecular assessments were performed, including in vivo arrhythmia provocation, action potential measurements in left ventricular cardiomyocytes and tissue slices, patch-clamp recordings from isolated left ventricular cells, and Western blot analyses.

Trained animals exhibited increased susceptibility to arrhythmias in vivo compared to sedentary controls. At the cellular level, cardiomyocytes from trained animals exhibited prolonged action potential duration, and enhanced action potential- and Ca^{2+} transient alternans. Additionally, sarcoplasmic reticulum Ca^{2+} content was reduced, while Ca^{2+} buffering capacity was increased, indicating remodelling of calcium dynamics. Western blot analysis revealed downregulation of CACNA1C (L-type Ca^{2+} channel) expression.

These findings suggest that chronic intensive physical training induces electrical and calcium handling remodelling in the canine heart, promoting the development of cardiac alternans. This proarrhythmic substrate may underlie the increased susceptibility to ventricular arrhythmias observed in elite athletes. Overall, our results provide novel insights into the mechanisms underlying malignant cardiac arrhythmias and sudden cardiac death in competitive athletes.

Reasons for the lack of action potential prolongation by the I_{Kr} blocker amsacrine

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Keywords: Amsacrine, Cardiac electrophysiology

Amsacrine is an antineoplastic agent used in acute leukemia. QT interval prolongation, ventricular fibrillation, and sudden cardiac death have also occurred during its use. The molecule contains a methanesulfonamide group just as in some inhibitors of the rapid delayed rectifier potassium current (I_{Kr}). I_{Kr} is responsible for initiating late repolarization, its pore forming channel protein is hERG, targeted by class III antiarrhythmics. Inhibition of I_{Kr} prolongs the action potential (AP) and can cause early afterdepolarizations, increasing the risk of cardiac arrhythmias. In the case of amsacrine, it has been shown to inhibit expressed hERG channels, but native I_{Kr} and AP studies have not yet been performed, therefore we investigated the concentration-dependent effects of amsacrine on cardiomyocytes.

Experiments were performed at 37 °C in enzymatically isolated canine left ventricular cardiomyocytes, which is considered a good model of the human heart. APs were measured with sharp microelectrode technique with the application of 1 and 10 μM amsacrine.

Both 1 and 10 μM amsacrine caused a similar decrease in the AP Peak, the AP amplitude (APA), and the maximal rates of depolarization ($V+_{max}$), and early repolarization ($V_{Ph1}max$). The membrane potential difference between resting value and that recorded at 20% and 50% duration of the action potential duration at 90 % of repolarization (APD_{90}), and the maximal rates of late repolarization ($V-_{max}$) were all decreased. For the latter parameters, higher concentration caused larger changes. Interestingly no AP lenghtening effect was observed.

Based on our results, amsacrine induced reduction of the Peak, APA, and $V+_{max}$ likely caused by sodium current inhibition. Reduction of $V_{Ph1}max$ and $V-_{max}$ can be the result of the inhibition of transient outward and inward rectifier potassium currents, respectively. Lack of AP prolongation might be due to the simultaneous inhibition of I_{Kr} and late sodium and/or L-type calcium currents.

β-adrenergic regulation of l-type Ca^{2+} current and Na^{+}/Ca^{2+} exchange current in canine ventricular cardiomyocytes

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Keywords: cardiomyocyte, electrophysiology, β-adrenergic activation, L-type calcium current, sodium/calcium exchange current

β-adrenergic activation is known to robustly increase L-type Ca^{2+} current ($I_{Ca\text{-}L}$), however, its effect on the Na^{+}/Ca^{2+} exchange current (I_{NCX}) is controversial. Present study was designed to determine the relative contribution of the protein kinase A (PKA) and the calcium/calmodulin-dependent protein kinase II (CaMKII) pathways in regulating $I_{Ca\text{-}L}$ and I_{NCX} during ß-adrenergic receptor activation, mimicked by 10 nM isoproterenol (ISO), in isolated canine ventricular myocytes. Also, the relationship between $I_{Ca\text{-}L}$ and I_{NCX} was examined under various experimental conditions. $I_{Ca\text{-}L}$ and I_{NCX} were dissected by 1 μM nisoldipine and 0.5 μM ORM10962, respectively, using action potential voltage clamp (APVC) and allowing physiological intracellular Ca^{2+} cycling. Peak current density, mid-plateau current density and the current integral for both $I_{Ca\text{-}L}$ and I_{NCX} were significantly increased by 10 nM ISO. The ISO induced augmentation of the mid-plateau current density and the current integral for both $I_{Ca\text{-}L}$ and I_{NCX} were significantly reduced by pretreatment with the PKA inhibitor H-89 (3 μM), or the CaMKII-inhibitor KN-93 (1 μM), or the combination of them, before the application of ISO. The inhibitory potency of KN-93 and H-89 was approximately similar in size and was not additive, i.e. the simultaneous application of H-89 and KN-93 failed to augment inhibition. All actions induced by ISO, KN-93, H-89 and their combinations caused proportional changes in $I_{Ca\text{-}L}$ and I_{NCX}. Moreover, the ratio of mid-plateau $I_{Ca\text{-}L}$ and I_{NCX} density as well as the ratio of $I_{Ca\text{-}L}$ and I_{NCX} integral was 2:1 in all experimental groups. It is concluded that both $I_{Ca\text{-}L}$ and I_{NCX} are dominantly controlled via a PKA- and CaMKII- dependent pathway being coupled in series. The proportional changes observed between $I_{Ca\text{-}L}$ and I_{NCX} strongly suggest that there is an additional regulatory mechanism based on changes in intracellular Ca^{2+} concentration in the case of I_{NCX}.

Sex differences in canine ventricular electrophysiology

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Keywords: sex difference, action potential, canine ventricular cells

Sex differences in human ECG parameters all well known, but in dogs, which is very similar to human electrophysiology, the comparison of cellular electrophysiological parameters between male and female animals is scarce. Therefore our aim was to elaborate the sex differences of the action potential (AP) of isolated canine left ventricular cells.

Our previously recorded canine cardiac APs were collected and grouped based on the sex of the animal, the used extracellular solution (Krebs, BTY, or NTY) and the possible transmural origin (endo, mid, epi) established based on the shape of the AP. Then the parameters of the AP such as its duration at 20, 50, 75, and 90% of repolarization (APD_{20}, APD_{50}, APD_{75}, and APD_{90}, respectively), the early and late plateau phase (at 20 and 50% of the APD_{90}) potentials (Plateau20, Plateau20 amplitude (AMP), Plateau50, Plateau50 AMP), resting membrane potential (RMP), maximal rates of phase 0, 1, and 3, AP peak and amplitude values were compared between male and female animals. To avoid pseudo-duplication, the AP parameters of the cells within the groups obtained from the same animal were averaged and expressed as mean±standard deviation.

Mid cells obtained from 62 male and 74 female animals showed no statistical sex difference in any of the examined AP parameters when all three type of solution was pooled together. In Krebs solution females (n=27) had slightly more negative RMP values (males (n=23): -82.5±4.4, females: -86.6±5.1 mV) which was the opposite in BTY (males (n=18): -84.7±2.9, females: -82.6±3.0 mV). Irrespectively of the applied solution, in epi cells obtained from 19 male and 33 female animals the value of APD_{20} was higher in males (83.8±35.9 vs 54.4±40.2 ms) just as the early plateau potential values (Plateau20: 13.3±6.8 vs 7.2±9.3 mV, Plateau20 AMP: 95.5±7.3 vs 90.1±8.3 mV). Maximal rate of phase 0 was higher in females (226.1±63.8 vs 267.8±50.9 V/s) while the maximal rate of phase 1 was smaller in females (-14.5±3.8 vs -17.3±4.0 V/s). In endo cells obtained from 25 male and 38 female animals there was no sex difference in any of the tested AP parameters.

We expected to see longer APs in cells obtained from female dogs similarly to human data but we found no such difference. In mid and endo cells there were no sex differences in most or all tested AP parameters. In epi cells slight sex dependence was seen in the early AP phases only. The origin of these sex differences require further research, eg. comparison of individual ion currents.

Session IX.
Young Investigators

The Role of HCN Channels in the Function of the Female Reproductive System in a Rat Model of PCOS

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Keywords: hyperandrogenism, HCN channel, ivabradine,

Polycystic ovary syndrome (PCOS) is often associated with hyperandrogenism that affects 8–13% of women of reproductive age and is frequently accompanied by vitamin D deficiency. Age-dependent expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels has previously been demonstrated in the ovarian tissue of female rats.

The aim of our study was to investigate the presence and function of HCN channels in the reproductive organs of female rats, as well as to explore the effects of hyperandrogenism and vitamin D supplementation on these channels.

A total of 52 female Wistar rats were divided into 4 groups: Control (C), Testosterone-treated (T), vitamin D-supplemented (D), and Testosterone+vitamin D (T+D). The control animals were kept under standard conditions, while the treated groups received transdermal testosterone and/or oral vitamin D supplementation for 8 weeks. After deep anesthesia, ivabradine-induced relaxation of the myometrium was examined ex vivo using wire myography; in the remaining tissues, immunohistochemical staining was performed for HCN1–4 channels, followed by determination of their optical density.

Based on our myography results, the non-pregnant myometrium responded to ivabradine with only weak relaxation, which was further reduced by chronic testosterone treatment (three-way ANOVA; testosterone effect 8.08%, $p = 0.009$). According to our histological examinations, HCN-1 density was lower in both vitamin D-treated groups compared to the control (T+D: 0.22±0.003, D: 0.22±0.005 vs. C: 0.23±0.005, $p<0.05$). HCN4 density was significantly reduced by testosterone (two-way ANOVA; 21.5%, $p = 0.0041$). In the ovary, the densities of HCN1–3 channels were evaluated according to the developmental phases of the follicles. The density of all three channels showed a significant correlation with follicle type (GLMM: $p<10–14$). Testosterone generally increased HCN1 density (GLMM: $p=0.012$), while the effect of vitamin D was mediated by the follicular developmental stage. HCN4 was present exclusively in oocytes, and its expression decreased following testosterone treatment (two-way ANOVA; 34.49%, $p<0.001$).

Our results confirm that HCN channels are present in the myometrium and the ovaries, and their expression is modulated both by hyperandrogenic state and vitamin D supplementation. The hyperandrogenic state reduces ivabradine-induced relaxation of the myometrium.

Kv1.5 channel dysfunction in pulmonary arterial hypertension

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Keywords: pulmonary arterial hypertension, potassium channels, ion channel dysfunction, vascular physiology, channelopathy

Pulmonary arterial hypertension (PAH) is a progressive vascular disease with a various genetic backgrounds including ion channels such as Kv1.5. Dysfunction of this channel was linked to the pathogenic mechanism of PAH as it regulates pulmonary arterial smooth muscle cell (PASMC) membrane potential and proliferation. However, the functional role of rare variants remains unclear.

Targeted sequencing of 540 PAH patients in this study found 11 rare Kv1.5 variants (8 novel). Six were functionally tested using electrophysiology and PASMC proliferation assays. Heterozygosity was modeled with tandem concatemers to mimic the patient-relevant condition.

Only the G435R variant showed a marked loss-of-function, with a 87±1.3% reduction in current amplitude. Single-channel recordings revealed a significantly shortened mean open time compared to WT (12.9±0.7 ms vs. 27.2±3.8 ms), indicating impaired channel gating. Western blot showed similar Kv1.5 expression in WT and G435R, ruling out reduced expression. Heterozygous concatemer constructs also demonstrated reduced current amplitudes (53±3% of WT), consistent with haploinsufficiency. Structural modeling localized the variant to the channel gate, supporting disrupted gating mechanism. In PASMCs, WT Kv1.5 overexpression suppressed proliferation, while G435R expressing cells failed to do so and showed enhanced proliferation compared to WT.

In conclusion, we identified a novel Kv1.5 variant with consistent loss-of-function across complementary electrophysiological and cellular assays, supporting its reclassification from variant of uncertain significance (VUS) to likely pathogenic.

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Uncovering an lpa-mediated immune escape mechanism in human melanoma

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Keywords: melanoma, lysophosphatidic acid, immunotherapy

Background: Lysophosphatidic acid (LPA) is a bioactive lipid mediator produced by melanoma. LPA regulates the release of various cytokines and chemokines from tumor cells, which in turn influence cancer progression, metastasis, and tumor immunity. While immune checkpoint inhibitors revolutionized the treatment of metastatic melanoma, approximately half of patients fail to respond favorably. Low expression of human leukocyte antigen-DR (HLA-DR) in melanoma is associated with poor prognosis and reduced responsiveness to anti-PD1 immunotherapy.

Aims: Because increased interleukin 10 (IL-10) levels are often associated with poor prognosis in various cancer types we investigated the potential role of LPA-induced cytokine release in the regulation of HLA-DR expression and clarify the underlying signaling mechanisms in human melanoma cells.

Methods: HEK293T cells transfected with the human DR6 promoter construct were used to measure the promoter activity induced by LPA. Evaluation of LPA-mediated signaling pathways in A375 and A2058 human melanoma cells was done using pharmacological inhibitors (Pertussis toxin, AM095, Ki16425), siRNAs (targeting NF-κB1, DR6, and IL-10), and neutralizing antibodies (anti-IL-10 and isotype control IgG1). LPA-induced gene expression was measured by quantitative RT-PCR whereas, cytokine release was detected by ELISA and expression of cell surface markers was measured by flow cytometry.

Results: Our results showed that LPA suppresses HLA-DR expression in human melanoma cells by upregulating the expression of death receptor 6 (DR6). DR6 is expressed in tumor cells and regulates diverse cellular functions, including cytokine release. Our results revealed that stimulation of the LPA receptor subtype 1 (LPAR1) by LPA increased DR6 expression via activation of NF-κB1 in human melanoma cells. Subsequently, LPA upregulated the expression and release of IL-10 via the LPAR1-DR6 axis, resulting in diminished expression of HLA-DR. Statistical analysis of the human melanoma transcriptome databases showed a significant correlation ($p<1.3x10-5$) between the expression of LPAR1, NF-κB1, DR6, and IL-10. Furthermore, an association between increased expression of LPAR1 and reduced effectiveness of anti-PD1 immunotherapy was found.

Conclusion: Taken together, our results indicate that the LPAR1-DR6-IL-10-HLA-DR autocrine signaling pathway constitutes a novel mechanism used by melanoma cells to evade immunosurveillance, providing resistance to anti-PD1 immunotherapy.

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Neutrophil EVs Modulate Monocyte Viability and Immune Responses

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Keywords: extracellular vesicles, neutrophils, monocytes

Introduction: Extracellular vesicles (EVs) are released by every known cell type, and through the transfer of bioactive molecules to recipient cells, representing crucial mediators of intercellular communication. Neutrophils (PMNs) contribute to immune regulation not only through direct cellular responses but also via EV release. Our group previously characterized three biologically distinct PMN EV populations released from resting, apoptotic and activated neutrophils. Our earlier studies showed that these PMN EVs exert either anti- or proinflammatory effects on neutrophils.

Aim: We investigated how different PMN EV subtypes influence monocyte viability and immune responses.

Methods Human neutrophils and monocytes were isolated from peripheral blood of healthy donors. PMN EVs were generated spontaneously (spEV), during apoptosis (apoEV) or following activation with opsonized zymosan (ozEV), then isolated by differential centrifugation and filtration. Monocyte viability was assessed by flow cytometry and lactate dehydrogenase (LDH) release. Functional responses were analyzed by ROS production, cytokine secretion and surface CD11b expression. RNA samples from EV-treated monocytes were collected for downstream transcriptomic analysis.

Results: ApoEV significantly improved monocyte viability, suggesting a protective effect on cell survival. In contrast, spEV exerted anti-inflammatory effects, including delayed ROS production, reduced LPS-induced IL-8 secretion and decreased CD11b surface expression. Preliminary RNA analyses further support EV-driven transcriptional reprogramming involving pathways related to viability, inflammation and immune regulation.

Conclusion: As central mediators of inflammation, neutrophils also shape immune responses through EV release. The observed anti-inflammatory effects of PMN EVs on monocytes indicate a potential role in promoting the resolution phase of inflammation.

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Mapping neutrophil migration induced by formyl peptides using novel biosensors

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N-terminally formylated peptides are potent chemoattractants that guide neutrophil migration. They have classically been proposed to originate from bacterial proteins and can also arise from mitochondrial proteins released by damaged eukaryotic cells, allowing them to act as both pathogen- and damage-associated molecular patterns through formyl peptide receptors, primarily FPR1. Although downstream signaling induced by these ligands is well characterized, direct real-time visualization of formyl-peptide release and spread remains limited.

Here, we aimed to develop a fluorescent biosensor capable of monitoring formyl-peptide release and spread during tissue injury and infection. To enable real-time detection, we engineered genetically encoded fluorescent biosensors by inserting a conformation-sensitive fluorescent protein into human FPR1. Screening approximately 260,000 variants in two rounds identified sensors with progressively improved performance, achieving up to ~400% fluorescence increase upon ligand binding. These sensors detected fMLF at concentrations as low as 5 nM, supporting their suitability for *in vivo* applications.

To investigate the effects of formyl peptides on neutrophils, we used complementary *ex vivo* and *in vivo* systems. Human and murine neutrophils were stimulated *ex vivo* with the canonical FPR1 agonist N-formyl-Met-Leu-Phe (fMLF) and with a photoactivatable fMLF derivative that we synthesized, enabling analysis of neutrophil polarization, chemotaxis, and associated Ca^{2+} signaling. In addition, we generated “humanized” zebrafish expressing human FPR1 specifically in neutrophils, which enhanced neutrophil responsiveness to fMLF *in vivo.*

Together, these tools provide an integrated platform for studying the spatiotemporal inflammatory roles of formyl peptides in living systems.

Improving the outcome of ischemic stroke through the muscle-brain axis

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Keywords: acute ischemic stroke, muscle stimulation, muscle-brain axis

Introduction: Acute ischemic stroke (AIS) is the second leading cause of death and third leading cause of disability worldwide. Nearly 50% of patients develop permanent hemiplegia, resulting in immobilization and muscle atrophy. Disruption of muscle–brain signaling may further impair neurological recovery. Our goal is to use EMS therapy to mobilize skeletal muscles and improve AIS outcomes.

Methods: Female and male C57BL/6 mice (n = 22) underwent acute ischemic stroke induction via 60-minute middle cerebral artery occlusion under isoflurane anesthesia. Sensorimotor deficits were evaluated daily for 72 hours using the Garcia Neurological Scale (GNS). Animals received daily electrical muscle stimulation (EMS) therapy (2 × 15 min sessions at 4 Hz). After 3 days, infarct volume was quantified by TTC staining, and mRNA expression in the quadriceps muscles and brain tissue was analyzed by RT-PCR.

Results: EMS therapy significantly reduced neurological deficits at 72 hours compared with untreated stroke animals (EMS stroke vs. stroke: 15 ± 2 vs. 12 ± 3 points). EMS-treated animals also exhibited a marked reduction in infarct volume (EMS stroke vs. stroke: 29.6 ± 12.1% vs. 45.1 ± 10.6%). In skeletal muscle samples, EMS altered the mRNA expression of key cytokines (*Tgf-1, Il-1*) and heat shock proteins (*Hsp25, Hsp70*). Similarly, in brain tissue, EMS treatment modulated the expression of inflammatory cytokines (*Tnf*, *Il1-β*) and the lactate transporter MCT1.

Discussion: Based on our results, EMS therapy exerts a protective effect in the animal model of AIS. The mechanism underlying this effect remains unclear; in our future experiments, we plan to employ multiplex serum proteomics and multimodal assessment of cerebral perfusion.

Session X. Clinical Cardiovascular Physiology: MHT-MÉT

Evidences supporting the measurement of carotid-femoral and brachial-ankle pulse wave velocity in clinical practice: teaser before the incoming Artery Society guidelines

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In the last two decades increasing number of evidence were cumulating supporting that the measurement of pulse wave velocity (PWV) has additional value in the management of patients with different cardiovascular diseases, especially in hypertension. Besides the „gold standard" carotid-femoral, brachial-ankle PWV also completes many requirements of a potentially useful cardiovascular biomarker. However, the measurement of PWV in the clinical practice is rare. Artery Society aimed to catalyze the penetration of these measurements into clinical practice with a new guideline which summarizes evidences in different conditions and also provide threshold limits of PWVs when organ damage can be considered. The speaker, who is a co-author of this incoming paper summarizes the cumulating evidences and provide a teaser into the structure and the content of the new guidelines.

Frequency and predictive factors of white-coat hypertension in grade 1 hypertensive patients: results of the Hungarian ABPM registry

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Current ESH guidelines recommend home and/or ambulatory blood pressure monitoring (ABPM) when white-coat hypertension (WCH) is suspected, particularly in people with grade 1 hypertension (gr. 1 HT). Our aim was to assess the prevalence of WCH in patients with gr. 1 HT defined by office BP measurement, and to determine the relevant predictive factors in WCH compared to sustained hypertension. Data were collected from the Hungarian ABPM Registry, which is an ongoing, multicenter, open-label, observational study. Adult patients (n=62,194, age: 56.15±15.18 years, female: 54.5%) with known or suspected hypertension were included. Validated Meditech ABPM-06 was used. Data analyzed in this report were collected between 01.03.2021-17.11.2025, we focused only on gr. 1 HT patients (n=28,198, 45.3%) who has similar patient characteristics (age: 55.76±15.30 years, female: 52.7%). The suspicion of WCH was the indication of ABPM in 11.3% (n=3,182) of the gr.1 HT patients. 69.8% (n=19,682) of gr. 1. HT patients were on antihypertensive treatment. Based solely on the 24-hour ABPM results WCH was diagnosed in 33.1% (n=9,331) of the gr. 1 HT patients. 74.3% (n=6,929) of them were treated, defined as white-coat uncontrolled hypertension (WUCH) and 25.7% (n=2,402) were untreated. The proportion of WCH patients was 20.8% (n=5,868) if we considered 24-hour, daytime and nighttime averages as well. In this case the proportion of WUCH patients was 73.9% (n=4,338). Female sex, age, hypercholesterolemia and physical inactivity increased the odds of WCH compared to sustained hypertension in patients with gr. 1 HT based on the ABPM results (see table below). In conclusion, if we consider all the three ABPM averages (24-hour, daytime and nighttime), the proportion of WCH patients was twice, if we use only the 24-hour averages it was three times higher than it was suspected according to the indication of ABPM. Assessing exclusively 24-hour BP average may lead to the overestimation of WCH. Female sex, age, hypercholesterolemia and physical inactivity were significant predictive factors of WCH compared to sustained hypertension in patients with gr. 1 HT.

Gr. 1 HT patients (n=28198)	OR	95% C.I. for OR	
		Lower	Upper
Female sex	**2.09**	**1.97**	**2.22**
Age	**1.02**	**1.01**	**1.02**
Hypercholesterolemia	**1.09**	**1.02**	**1.16**
Coffee consumption	**0.86**	**0.80**	**0.92**
Snoring	**0.65**	**0.61**	**0.70**
Physical inactivity	**1.17**	**1.08**	**1.28**
Smoking	**0.89**	**0.81**	**0.99**
Obesity	**0.58**	**0.54**	**0.62**

Cardiovascular diseases after kidney transplantation: translating traditional risks into precision prevention

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Keywords: kidney transplant, cardiovascular risk, surrogate

Cardiovascular disease (CVD) is the leading cause of morbidity and death among kidney transplant (KT) recipients with functioning graft. Although successful transplantation substantially reduces cardiovascular risk compared with remaining on dialysis, KT recipients continue to face higher relative risk of cardiovascular death compared to the general population. The multifactorial pathogenesis of post-transplant CVD encompasses baseline comorbidities (diabetes, hypertension, pre-existing atherosclerosis), dialysis-induced vascular damage, graft dysfunction, post-transplant diabetes and dyslipidaemia, as well as the direct and indirect cardiometabolic effects of contemporary immunosuppressive regimens. Emerging risk-stratification paradigms integrate clinical scores with non-invasive assessments of subclinical vascular and metabolic injury, including carotid intima–media thickness, pulse wave velocity and other arterial stiffness indices, alongside body-composition–based phenotyping of sarcopenic obesity and visceral adiposity. Novel preventive and therapeutic concepts—ranging from SGLT2 inhibition and intensified lipid and blood pressure control to more individualized immunosuppression—are reshaping post-transplant cardiovascular care. In this summary presentation, framed within a translational medicine context, these advances are used to delineate critical gaps and to open concrete avenues for experimental research targeting vascular remodelling, immunometabolic pathways and precision cardiovascular risk modification in kidney transplantation.

Sympathetic overactivity: renal denervation in resistant hypertension

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Keywords: hypertension 1, renal denervation 2, sympathetic nervous system 3

The sympathetic nervous system plays a pivotal role in the pathophysiology of primary hypertension. The kidney functions as both a driver and a mediator of sympathetic overactivity through inflammatory and genetic pathways. Renal denervation (RDN) is a contemporary, catheter-based procedure that facilitates blood pressure reduction through the targeted ablation of sympathetic nerve fibres located along the renal arteries, specifically for uncontrolled hypertensive patients.

Modern renal denervation has transitioned from limited main-artery ablation to a comprehensive distal strategy, utilizing either multi-electrode radiofrequency catheters or circumferential ultrasound systems to target nerves where they lie closest to the vessel lumen. Clinical evidence, including the SPYRAL HTN-ON MED and long-term 10-year follow-up studies, demonstrates that increasing ablation density to approximately 44 points per patient significantly enhances norepinephrine depletion and ensures sustained blood pressure reduction.

The longest follow-up study to date demonstrates that RF-RDN provides a sustained and significant reduction in blood pressure, with a 16.2 mmHg decrease in 24-hour mean systolic values maintained even ten years post-procedure. Furthermore, the intervention proved highly effective in the long term, as the proportion of patients achieving a systolic blood pressure below 140 mmHg increased from 18% at baseline to 68% after a decade.

Real-world data indicates that RF-RDN is a safe procedure, with major complications such as significant renal artery stenosis or dissection—including cases requiring stenting—occurring at a very low frequency of only 0.20% per year.

In summary, the evolution towards high-density distal ablation has established renal denervation as a safe and potent therapeutic option for long-term blood pressure management. With proven efficacy and a remarkable safety profile sustained over ten years, RDN represents a robust non-pharmacological pillar for patients with uncontrolled hypertension.

Age- and sex-specific haemodynamic mechanisms underlying different hypertensive phenotypes

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Background: Essential hypertension is approached as a uniform condition; however, it comprises distinct blood pressure phenotypes – namely isolated systolic, systolic–diastolic, and isolated diastolic hypertension (ISH, SDH, and IDH, respectively) – that vary in prevalence according to age and sex. We hypothesized that these differences reflect underlying variations in hemodynamic mechanisms.

Methods: In this cross-sectional analysis of the ACCT (Anglo-Cardiff Collaborative Trial), 5,371 individuals (2,402 men), aged 18–92 years, free of cardiovascular disease and medication, were included. Blood pressure, cardiac output (CO), stroke volume (SV), peripheral vascular resistance (PVR), and aortic pulse wave velocity (aPWV) were measured. Participants were stratified by sex and age (<30, 30–60, and >60 years), as well as by blood pressure phenotype based on clinic (seated) blood pressure measurements.

Results: ISH was the most common hypertensive phenotype in young men and was characterized by elevated CO and SV. In contrast, SDH and IDH were more prevalent in younger females, with SDH associated with elevated PVR and aPWV, and IDH with increased PVR. SDH was the most common phenotype in middle age and was accompanied by increased PVR in both sexes. In older individuals, ISH was again the most common hypertensive phenotype. However, unlike in younger adults, it affected both men and women equally (~1:1) and was characterized by elevated aPWV and PVR.

Conclusions: Hypertensive phenotypes are associated with distinct hemodynamic profiles that vary according to age and sex. Tailoring treatment to the predominant underlying hemodynamic abnormalities could lead to more effective interventions in essential hypertension.*

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Session XI.
Translational cardiology

Myotropic therapy and the regulation of myocardial function

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Introduction: Cardiomyotropic agents modulate myocardial contractility through the direct alteration of the myosin–actin interaction, via a calcium-independent mechanism. Omecamtiv mecarbil (OM) and danicamtiv (DN) act as myosin activators, whereas aficamten (Af) and mavacamten (Mava) function as myosin inhibitors. However, their clinical efficacy and therapeutic profiles differ substantially.

Methods: We investigated the contractile and hemodynamic effects of these agents in human membrane-permeabilized cardiomyocytes and in rat models. Echocardiographic assessments and intracellular calcium dynamics enabled a comprehensive characterization of systolic and diastolic function.

Results: Myosin activators and inhibitors exhibited distinct contractile and hemodynamic profiles, which did not consistently translate into clear clinical benefit. Marked differences were observed among the agents regarding their effects on systolic and diastolic parameters, as well as on compensatory mechanisms.

Conclusion: The effects of myotropic agents are complex and highly dependent on clinical context; direct modulation of contractility alone is not predictive of therapeutic outcome. The compensatory mechanisms observed with myosin inhibitors warrant particular attention regarding their long-term clinical significance.

SGLT2 inhibitors in cardioprotection: evidence across species and underlying mechanisms

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Keywords: SGLT2 inhibitors, cardioprotectiton, cardiac remodeling

Cardiovascular disease (CVD) and cancer remain the leading causes of mortality worldwide. Advances in metabolic pharmacotherapy—particularly sodium-glucose co-transporter 2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1 RA)—have significantly transformed the management of metabolic and cardiometabolic disorders. These agents have demonstrated robust clinical efficacy, including reductions in cardiovascular mortality and heart failure (HF) hospitalizations, thereby redefining contemporary therapeutic strategies. Beyond their established benefits in HF, accumulating evidence, including our own investigations, supports a broader spectrum of cardiometabolic and systemic effects. SGLT2i exhibit potential in attenuating cardiomyopathy associated with Duchenne muscular dystrophy and in promoting regression of left ventricular hypertrophy in preclinical and clinical setting likely through mechanisms involving improved myocardial energetics, modulation of inflammatory and fibrotic pathways. Concurrently, there is increasing interest in their role within oncology, with preclinical and emerging clinical data suggesting potential effects on tumor biology and mitigation of cancer therapy–related cardiovascular toxicity. Collectively, these observations indicate that SGLT2i and GLP-1 RA exert pleiotropic effects that extend beyond glycemic control, impacting multiple organ systems. By the end of this presentation, participants will have developed a mechanistic and translational understanding of the expanding therapeutic roles of SGLT2i beyond traditional metabolic indications. They will be able to critically appraise the underlying biological pathways mediating their cardioprotective and potential anticancer effects, and to evaluate emerging evidence supporting their integration into future therapeutic paradigms.

Development of miRNA therapeutics for cardioprotection

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Keywords: cardioprotection, miRNA, oligonucleotide, multitarget drug

Pharmacological management of myocardial infarction and the subsequent development of post-infarction heart failure remains a major unmet clinical need, most likely due to the highly complex underlying molecular mechanisms. Consequently, therapeutic strategies that target entire molecular networks rather than single molecular entities may offer a more effective approach to cardioprotection. Unbiased and comprehensive analyses of gene expression patterns in healthy and diseased myocardium can facilitate the mapping of transcriptomic and proteomic networks, enabling the identification of novel molecular targets for cardioprotective interventions.

MicroRNAs (miRNAs), non-coding oligonucleotides that simultaneously regulate multiple mRNA transcripts, constitute dynamic regulatory nodes within these transcriptomic networks. Targeted modulation of such networks using miRNA-based oligonucleotide therapeutics represents a promising strategy for treating diseases driven by complex molecular pathophysiology.

Through systematic, unbiased analysis of the transcriptomic networks in healthy, infarcted, and conditioned myocardial tissue, we identified several endogenous cardioprotective miRNAs. These candidates were subsequently validated in vitro and in vivo. We designated this class of cardioprotective miRNAs as *protectomiRs*. To support further discovery, we are developing a miRNA identification platform that integrates specialized bioinformatic software tools (see miRNAtarget.com) to reconstruct transcriptomic and proteomic molecular networks and to predict oligonucleotide drug candidates for myocardial infarction and heart failure.

In summary, miRNA-based therapeutics may offer a transformative opportunity for treating diseases characterized by complex molecular mechanisms, including myocardial infarction. We have developed a platform that enables systematic miRNA discovery and are currently advancing a pipeline of miRNA-based therapeutic compounds toward clinical development across multiple indications.

The role of apelin as an exerkine in cardiac and skeletal muscle exercise adaptation

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Keywords: apelin, exerkine, adaptation to exercise

The role of apelin in cardiac and skeletal muscle adaptation to exercise remains incompletely understood. Using murine models, we demonstrated that apelin deficiency (KO) induces a maladaptive response to high-intensity interval training (HIIT). While wild-type mice develop mild concentric cardiac hypertrophy, apelin KO mice exhibit eccentric ventricular remodeling. In skeletal muscle, apelin deficiency promotes a fiber-type shift toward smaller, slow-twitch type I fibers, accompanied by downregulation of glucose metabolism–related genes and upregulation of fatty acid transport markers. Mechanistically, these changes are associated with impaired activation of insulin-like growth factor-1 (IGF-1) signaling, resulting in a phenotype resembling aged muscle.

Complementary human studies in professional athletes show that plasma apelin-13 levels increase transiently following maximal exercise, with marked interindividual variability. Importantly, exercise-induced changes in apelin-13 correlate positively with maximal oxygen uptake, metabolic equivalents, and peak circulatory power.

Collectively, these findings identify apelin as a key exerkine required for physiological muscle adaptation. Its deficiency recapitulates features of premature aging and sarcopenia, whereas its exercise-induced elevation is associated with superior cardiopulmonary performance. Accordingly, modulation of apelinergic signaling may represent a promising therapeutic strategy to counteract age-related muscle decline and improve cardiorespiratory fitness.

Cell-matrix interactions in cardiovascular disease

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Endothelial cells regulate cardiovascular homeostasis through interactions with the extracellular matrix. This presentation examines endothelial cell-matrix interactions in cardiovascular remodelling in dilated cardiomyopathy and microvascular disease using human induced pluripotent stem cell (hiPSC) models. To investigate endothelial-specific contributions, we generated arterial and cardiac microvascular-like endothelial cells through scalable 2D and 3D protocols. Single-cell RNA sequencing confirmed cardiac and microvascular gene signatures with phenotypic stability and angiogenic potential, alongside pericyte-like cells suitable for disease modelling and endothelial-targeted studies. The hiPSC-endothelial cell platform combined with patient-derived matrices provides a tool for studying cell-matrix interactions and enables high-throughput screening of therapies targeting endothelial dysfunction. As an example, we engineered melt electrowritten scaffolds that support maturation of hPSC-cardiomyocytes and assembly of synchronously beating multi-layer patches. Inclusion of endothelial cells promoted vascular regeneration post-myocardial infarction in mice through microvascular support. To study endothelial-ECM coupling in non-ischemic cardiomyopathies, we combined human explant histopathology, targeted matrix proteomics, and functional assays of control and LMNA-mutant hiPSC-derived endothelial cells seeded on decellularized human cardiac ECM. Our findings demonstrate that endothelial dysfunction in LMNA-DCM extends beyond cardiomyocyte pathology and involves aberrant endothelial-ECM crosstalk that promotes fibrosis and microvascular rarefaction. These findings indicate the potential to restore vascular homeostasis and reduce maladaptive remodelling in cardiomyopathies and ischemic conditions.

Increased electrical instability driven by action potential duration and amplitude alternans in human heart failure: modulation by selective NCX inhibition

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Keywords: heart failure, remodelling, alternans, arrhythmia, action potential, NCX

Heart failure (HF) is a complex, progressive disease characterized by structural and electrical remodeling of the heart, significantly increasing the risk of ventricular fibrillation (VF). However, prior to the onset of VF, a potentially reversible phenomenon known as alternans may develop. Action potential (AP) alternans refers to beat-to-beat oscillations in AP duration (long–short), leading to repolarization inhomogeneity. As no specific pharmacological intervention currently exists to prevent alternans, this study investigated the effects of the selective Na^+/Ca^{2+} exchanger (NCX) inhibitor ORM-10962 on alternans formation in both failing and non-failing human hearts.

Tissue samples from failing and non-failing human hearts were used. HF samples were obtained from the Városmajor Heart and Vascular Centre, while non-failing tissues were collected from donor hearts. APs were recorded using conventional microelectrode techniques in left and right ventricular preparations.

In HF samples, AP duration (APD) alternans was significantly larger than in non-failing hearts. In several cases, pronounced amplitude alternans were observed in left ventricular HF preparations, predominantly at higher pacing rates. These further increased APD alternans magnitude and ultimately led to 2:1 conduction block. Preparations exhibiting amplitude alternans also showed slower AP amplitude restitution kinetics. Treatment with 1 μM ORM-10962 shortened APD in HF samples, reduced APD alternans magnitude, and shifted the onset of amplitude alternans and conduction block to higher pacing rates.

Electrical remodeling in HF markedly increases APD alternans, which appears to worsen with age, possibly due to progressive SERCA downregulation. Notably, failing hearts may develop amplitude alternans at higher heart rates, further amplifying APD alternans. The underlying mechanism may involve impaired recovery of the sodium current. Selective NCX inhibition attenuates alternans in the failing human heart, likely through APD shortening and its effects on calcium handling.

Session XII.
Translational immunology

Our recent results regarding the use and mechanisms of modulated electro-hyperthermia (mEHT) in a triple-negative breast cancer (TNBC) mouse model

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Introduction: Following an enthusiastic interest in hyperthermia for cancer treatment, there was a strong setback after the failure of clinical trials in the 1990s. The main reasons were that cancer is not selectively sensitive to hyperthermia, and the lack of a therapeutic window with homogenous heating.

Material and Method: Building on these experiences, modulated electrohyperthermia utilizes the autofocusing of the electromagnetic field due to altered lipid rafts (at 13, 5 MHz) and cancer metabolism, leading to effective focusing on the cancer tissue. The synergistic effects between heat and the electromagnetic field - also used in tumor treating fields (TTF) - result in significant cancer tissue damage. mEHT is used in the clinics in ca. 30 countries as a complementary treatment. A phase III randomized clinical trial of ovarian cancer demonstrated a doubling of disease-free survival (DFS) (14% standard of care, 32% addition of mEHT) at 5 years and an abscopal effect on extrapelvic metastasis (complete metabolic resolution on PET/CT).

Result and Discussion: To understand the driving mechanisms induced by mEHT, we continuously improve the rodent applicator (LabEhy), including the electrode used for the treatment, the modulation software, the skin cooling system, and the mode of application. In a triple-negative breast cancer mouse model (4T1 in Balb/C), we performed a series of multiplex studies and explored the main pathways activated by mEHT. Based on these data, we have demonstrated synergism between mEHT and inhibition of 1) the heat-shock response, 2) hypoxia inducible factor (HIF1a) mediated vasculogenesis, and 3) COX2 mediated inflammation. Currently, we investigate possible synergisms between mEHT and 4) anticancer immune mechanism enhancement (such as immune checkpoint blockade and the innate immune system).

Conclusion: We hope to be able to convert cold tumors to immune-infiltrated ones and thus extend the patient cohort for effective immune checkpoint blockade therapy. With the recent accumulation of evidence on the mechanisms and therapeutic combination strategies of mEHT its clinical applications are currently gaining momentum.

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Study of adverse cardiovascular side effects of Pfizer and Moderna mRNA-LNP COVID-19 vaccines in a rat hypersensitivity model

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Lipid nanoparticles (LNPs), including new mRNA-LNP vaccines, induce hypersensitivity reactions (HSRs) in patients. Although acute and chronic side effects are rare, they have affected many patients as billions of these vaccines have been administered. In our rat HSR model, we investigated the possible role of pseudoallergy, a common side effect of phospholipid-containing drugs.

Male Wistar rats were anesthetized with pentobarbital (90 mg/kg i.p.; n=6-9/group). Blood pressure (BP) was monitored through a femoral arterial cannula. Blood samples were taken at predetermined times. Plasma C3a and TXB2 concentrations were assayed by ELISA. Blood cell counts were measured using Abacus hematology analyzer.

Comirnaty and Spikevax vaccines were administered i.v. in 6:1 dose ratio. It caused slow-onset temporary hypotension lasting 40-50 minutes. The maximum drop in BP was 20-30 mmHg. Plasma TXB2 levels increased to 1.5-fold and recovered after 10-30 mins. Complement C3a increased by approx. 20% over 60 minutes. Leukocyte and platelet count slightly decreased. One week of pretreatment with Doxebo (1 mg/kg), a PEGylated empty liposome, resulted in a significant increase in IgG and IgM antibody levels. A much greater and longer-lasting BP response was observed, but plasma TXB2 and C3a concentrations and blood counts were only slightly elevated. The pseudoallergy symptoms of LNP-based vaccines were quantitatively different from those of conventional liposomal nanoparticles. The role of complement was minimal.

In summary: 1. Both COVID vaccines induced pseudoallergy in rats, Spikevax being more effective than Comirnaty; 2. Doxebo pretreatment enhanced side effects of the PEGylated mRNA-LNP vaccines.

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Characterization of the role of the local lymphatic vasculature in organ-specific responses induced by the nucleoside-modified mRNA-LNP platforms

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Keywords: immune responses, nucleoside-modified mRNA-LNPs, lymphatic vasculature

Lipid nanoparticle-encapsulated nucleoside-modified messenger RNA (mRNA-LNP) vaccines induce robust humoral immune responses; however, the underlying molecular and cellular mechanisms involved in this process are still not fully understood. The local lymphatic vasculature participates in several immune processes, yet its role in the mRNA-LNP vaccine-induced immunity remains unclear. Our aim was to investigate the role of the lymphatic vasculature in nucleoside-modified mRNA-LNP induced organ-specific responses in mouse models. We injected Dil fluorophore-labeled LNPs and eGFP-encoding mRNA-LNP complexes into wild-type or lymphatic-deficient mice and examined their uptake and expression using fluorescence immunohistology and flow cytometry. Influenza hemagglutinin encoding mRNA-LNPs were injected into mice with local lymphatic deficiency, and specific antibody titers were determined. Our results suggest that at least four lymphatic-dependent processes are involved in the mechanism of action of mRNA-LNPs. First, a subset of lymphatic endothelial cells expressed mRNA delivered by the LNPs. Second, mRNA-LNPs reached the lymph nodes and were taken up and expressed by subcapsular sinus macrophages. Third, dendritic cells efficiently internalized, transported, and expressed mRNA-LNPs. Fourth, neutrophils and macrophages internalized and transported LNP complexes but showed minimal mRNA expression. The mRNA-LNP vaccination into lymphatic-deficient regions resulted in a barely detectable humoral immune response suggesting that these processes are crucial for immunization. These findings demonstrate that the local lymphatic vasculature is essential for effective mRNA-LNP vaccine-induced humoral immunity and provide insight into optimizing the next-generation of mRNA vaccine platforms.

Investigation of the role of neutrophil granulocytes in the immune response induced by nucleoside-modified mRNA-LNP vaccines

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Keywords: nucleoside-modified mRNA-LNPs, neutrophils, immune response, vaccines

Nucleoside-modified, lipid nanoparticle-encapsulated mRNA (mRNA-LNP) vaccines induce strong humoral immune responses; however, the underlying molecular and cellular mechanisms remain incompletely understood, particularly the potential role of innate immune mechanisms.

Our aim is to investigate the role of neutrophils in the immune response elicited by nucleoside-modified mRNA-LNP vaccines in experimental mouse models.

To identify cells involved in mRNA-LNP uptake and expression, fluorescently labelled empty LNPs (Dil-LNP) and eGFP-encoding mRNA-LNPs were injected into the hind limb of wild-type and transgenic neutropenic mice. The injection site and draining lymph nodes were analyzed by stereomicroscopy and flow cytometry. To evaluate humoral responses, neutropenic and control mice were injected with influenza hemagglutinin-encoding mRNA-LNP, and serum anti-hemagglutinin IgG, IgM and neutralizing antibody titers were measured.

In wild-type mice, Dil-LNP and eGFP mRNA-LNP induced pronounced neutrophil infiltration in the draining lymph nodes. Neutrophils took up Dil-LNP to a greater extent than they expressed eGFP. Neutropenic mice displayed significantly fewer Dil- and eGFP-positive dendritic cells compared with littermate controls. Anti-hemagglutinin IgG titers were significantly lower in neutropenic animals, while specific IgM titers were elevated following vaccination.

Our results indicate that injection of the mRNA-LNPs triggers significant neutrophil recruitment. Neutrophils may influence antigen presentation via dendritic cells. Our results suggest that isotype switching is impaired in the absence of neutrophils. Taken together, these findings indicate that neutrophils may play an important role in the development of an effective adaptive immune response to mRNA-LNP vaccines. Our study enhances the understanding of mRNA-LNP vaccine mechanisms and may support platform optimization.

The circadian rhythm across the body: comparison of the central and peripheral clock functions

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Keywords: circadian rhythm, peripheral clocks, circadian phase variability

Background: Circadian rhythms enable organisms to anticipate and adapt to the rhythmic changes of the environment. In mammals, circadian rhythm coordination is organized hierarchically. The central pacemaker in the suprachiasmatic nucleus is well characterized at both the cellular and molecular levels. Although molecular components of the circadian clock have also been identified in several peripheral tissues, the functional significance of many peripheral oscillators remains unclear. Communication mechanisms between peripheral oscillators and the central clock are not fully understood, and alignment among peripheral clocks is completely unexplored. However, assessing the phase of tissue-specific rhythmic functions may be critical for effective chronotherapy.

Aims: The aim of this study was to investigate the relationship between the central clock and two peripheral clocks – peripheral blood mononuclear cells (PBMCs) and buccal mucosa (BM) – in humans.

Methods: Blood and buccal mucosa samples were collected from 12 healthy subjects at six time points over a 24-hour period. Leukocyte populations and plasma cortisol levels were measured, and RNA was isolated from PBMCs and BM. Clock gene expression (*PER2, REV-ERBα, DBP*) was analyzed using RT-PCR. Core body temperature (CBT) was continuously monitored using CALERA Research, and chronotype was assessed using the Munich Chronotype Questionnaire. Statistical analysis was performed in R.

Results: Significant daily oscillations of *REV-ERBα* and *DBP* were observed in both peripheral tissues. Leukocyte subpopulations, plasma cortisol levels, and CBT also exhibited rhythmic changes. Surprisingly, individual-level comparisons revealed antiphasic clock gene oscillations between PBMCs and BM. While peripheral clock acrophases showed marked interindividual variability, rhythm parameters influenced by the master clock were more consistent.

Conclusion: Our data suggest that social constraints may strongly shape the phase of the master clock. Clock gene expression in peripheral tissues shows tissue-specific phases and marked interindividual variability. These insights may serve as important considerations in the planning of chronotherapy.

Proteolytic products in the background of neutrophil granulocyte impairment in severe human sepsis

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Keywords: human sepsis, neutrophilic granulocytes

Background: Bacterial sepsis is a severe complication of infection with high mortality. Despite its significance, little is known about the function of neutrophilic granulocytes (PMNs), key cells in bacterial elimination, during sepsis.

Aims: We investigated circulating PMNs from septic patients, comparing their function with pathogen type, clinical status, and healthy controls. We also examined whether plasma factors from septic patients influence PMN function.

Methods: The study included 31 septic patients and 14 healthy volunteers. Disease severity was assessed using APACHE II. Blood samples were collected on days 1, 7, and 30. PMNs were isolated using Ficoll-Paque. Functional analyses included viability, phagocytosis, bacterial killing (against *Staphylococcus aureus* and *Escherichia coli*), and superoxide production. Naïve PMNs were incubated with septic plasma samples.

Results: Septic PMNs showed reduced maximal killing of *S. aureus*, decreased phagolysosomal superoxide production, and increased extracellular unstimulated superoxide, all correlating with disease severity. Viability, phagocytosis, and *E. coli* killing were unchanged. Functional impairments were transferable to naïve PMNs via a heat-stable, protease-resistant 3–12 kDa plasma fraction. These effects correlated with protein concentration and were reversible with healthy plasma. Protease treatment or CD18 inhibition blocked the effect, while DNase, lipase, saccharidase, heat, or acid treatment did not. Proteolytic products from blood samples induced similar dysfunction.

Conclusions: In sepsis, activated proteases likely generate peptides that impair neutrophil function, contributing to immune dysfunction.

Session XIII. Microcirculation in Neurological Diseases: MMVBT

PAQR5 membrane progesterone receptor regulates the blood–brain barrier during brain metastasis formation

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Keywords: blood–brain barrier, brain metastasis, membrane progesterone receptor

Brain metastases are a common and often fatal complication of certain cancer types, such as triple-negative breast cancer. However, the molecular pathways driving brain metastasis formation, including the migration of cancer cells from the bloodstream to the brain parenchyma across the blood–brain barrier, are not yet fully defined.

Therefore, using highly relevant mouse and human model systems, we investigated the mechanisms by which triple-negative breast cancer cells and their released extracellular vesicles modulate the blood–brain barrier-forming endothelium to increase its permissiveness to tumour cell entry into the brain. We observed that extracellular vesicles derived from tumour cells were taken up by cerebral endothelial cells, where they induced miR-146a-5p- and TGF-β1-mediated downregulation of PAQR5/mPRγ, a membrane progesterone receptor. This, in turn, led to disruption of interendothelial tight junctions, particularly through repression of claudin-5 expression, a critical protein for maintaining barrier function. As a consequence of BBB opening, cancer cells migrated in greater numbers across brain endothelial monolayers with reduced PAQR5 expression.

Altogether this identifies a novel mechanism by which triple-negative breast cancer-derived extracellular vesicles compromise blood–brain barrier integrity, thereby facilitating transendothelial migration of cancer cells and promoting brain metastasis development. Moreover, this study is the first to highlight the role of membrane progesterone receptors in regulating the blood–brain barrier.

Roles of NOS isoforms in the adaptation of the cerebrocortical circulation to unilateral carotid artery occlusion

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The investigation of cerebral autoregulation mechanisms has gained significant importance due to the rising prevalence of carotid artery stenosis globally. Our study aimed to examine the changes in cerebrocortical blood flow (CoBF) patterns and explore the involvement of pial collaterals in the adaptive response following unilateral carotid artery occlusion (CAO). Additionally, we assessed the role of nitric oxide (NO), with a particular focus on the different isoforms of nitric oxide synthase (NOS).

Experiments were performed on wild-type (WT), NOS1 KO, NOS3 KO, NOS1/3 DKO (NOS1 and NOS3 double knockout), and WT mice treated with the non-selective NO synthase inhibitor: L-NAME. The adaptational capability of cerebrovascular autoregulation was determined by analysing the regional changes in cerebrocortical blood flow (CoBF) using laser-speckle imaging after unilateral CAO. The morphology of the pial collaterals, connecting the frontoparietal and temporal regions, was also analysed.

Our results show that the Willis circle alone is insufficient to compensate immediately for the loss of one carotid artery, as a significant CoBF decrease can be seen in the ipsilateral temporal cortex in the acute phase of the adaptation. However, pial collaterals attenuate the ischemia of the temporal cortex at the expense of the blood supply of the frontoparietal region. Regarding the role of NOS isoforms, NOS1 KO and NOS3 KO animals show a partially preserved ability for adaptation, whereas in animals lacking both NOS1 and NOS3 isoforms, an impaired cerebrocortical blood flow adaptation to CAO can be determined, mainly in the subacute phase, which alteration can be attributed partly to the higher level of tortuosity in pial collateral vessels. The pharmacological inhibition of NOS isoforms with L-NAME results in severe decrease of CoBF values in the acute phase, in both hemispheres.

In conclusion, our experiments provide evidence for the major role of NO in cerebrovascular adaptation, involving both morphological and functional changes of the cerebrocortical microcirculation.

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Alzheimer-stroke continuum: the role of spreading depolarization

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Background: Cerebral amyloid angiopathy causes capillary obstruction in Alzheimer's disease (AD). Although AD is a neurodegenerative disorder, its microvascular alterations resemble capillary dysfunction observed after acute ischemic stroke (stroke). Nevertheless, the co-morbidity of AD and AIS remains poorly understood. Here we report a novel finding of increased spreading depolarization (SD) frequency in AD mice after stroke. Because SD is known to induce capillary constriction in stroke, we hypothesize that SD may accelerate AD progression.

Aim: To determine whether SD disrupts neurovascular coupling (NVC) and contributes to microvascular dysfunction in AD.

Methods: Aged (19–23 months) female and male APP/PS1 transgenic (AD, n=7) and wild-type (WT, n=8) mice were anesthetized with isoflurane (0.8–1%). Stroke was induced by transient (60 min) middle cerebral artery occlusion, followed by reperfusion. Three days post-stroke, infarct volume was assessed using T2- and diffusion-weighted MRI. Microvascular function was evaluated by whisker stimulation–evoked NVC measured with functional ultrasound imaging.

Results: AD mice developed more diffuse and expanded ischemic lesions and exhibited significantly more SDs in response to whisker stimulation compared to WT mice (13 vs. 3 SDs). This indicates heightened neuronal excitability in AD mice after stroke. Furthermore, functional hyperemia was reduced in AD mice (NVC: 9.3±5% vs. 15.5±3.1% in WT), suggesting impaired neurovascular function.

Conclusions: These findings align with clinical observations of neuronal hyperexcitability and epileptiform activity in AD patients. While seizures are considered precursors of SD, SD has not previously been observed in AD. We propose the role of SD in AD-related neurodegeneration.

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Disruption of vitamin D signaling impairs cerebrovascular adaptation to carotid artery occlusion

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Deficiency in vitamin D, a lipid-soluble steroid hormone, affects approximately 24% to 40% of the population in the Western world. In recent decades, it has been increasingly linked to the pathogenesis and progression of cerebrovascular diseases. Our aim was to investigate the impact of impaired vitamin D signaling on cerebral microcirculation, considering sex hormone status as it may also influence the prevalence of cerebrovascular disorders. We analyzed the anatomical and functional aspects of cerebrovascular adaptation to unilateral carotid artery occlusion (CAO) in intact male and female, as well as in ovariectomized and hyperandrogenic female mice, with either normal or functionally inactive vitamin D receptor (VDR). Cerebrovascular adaptation was assessed by analysing changes in cerebrocortical blood flow using laser-speckle imaging following CAO. In addition, the morphology of leptomeningeal anastomoses was evaluated. Cerebrocortical blood flow showed a significantly increased drop and delayed recovery after CAO in male mice with a functionally inactive VDR. This was associated with a reduced number and increased tortuosity of pial anastomoses. In contrast, in female mice, ablation of VDR alone did not impair cerebrovascular adaptation to CAO despite the reduced number of pial collaterals. Surprisingly, ovariectomy did not exacerbate the effects of disrupted VDR signaling. However, androgen excess combined with VDR inactivity resulted in prolonged hypoperfusion in the cerebral cortex ipsilateral to the occlusion. In conclusion, our findings suggest that the cerebrovascular consequences of disrupted VDR signaling are less pronounced in females than in males, indicating a level of protection in females even after ovariectomy. Conversely, even short-term androgen excess in the absence of VDR signaling may lead to vasoregulatory dysfunction, potentially contributing to the increased risk and severity of cerebrovascular diseases.

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Session XIV. Signaling Pathways in Physiological and Pathophysiological Regulation

C3a signaling: Bidirectional vasoactive effects in angiotensin II–driven vascular dysfunction and ischemic heart disease

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Keywords: complement, anaphylatoxin, inflammation

Background: The complement anaphylatoxin C3a is a potent mediator of vasoconstriction. This study examined how inflammatory conditions - chronic angiotensin II (Ang II) in mice and ischemic heart disease (IHD) in humans - modify C3a-induced vasoconstriction.

Methods: Isometric tension was assessed by myography in aortic segments from C57BL/6 mice and in human coronary arteries. Human samples were obtained from IHD patients and non-ischemic controls. Mice received Ang II (520ng/kg/min) or saline via osmotic minipumps for two weeks. In selected experiments, CD11b-DTR mice underwent diphtheria toxin-mediated depletion of CD11b+ cells. C3AR1 expression was assessed by qPCR and immunohistochemistry; cell-type specificity was analyzed via public single-cell RNA-seq data.

Results: Aortas from Ang II-treated mice and vessels from IHD patients showed enhanced C3a responsiveness and increased C3AR1 expression, predominantly in the adventitia. Endothelium removal augmented C3a-induced contraction, whereas adventitia removal or CD11b+ cell depletion abolished the response. Cyclooxygenase and thromboxane prostanoid (TP) receptor inhibition attenuated C3a responses in both control and Ang II-treated vessels.

Conclusions: Systemic inflammation amplifies C3a-induced vasoconstriction via thromboxane A2 release from CD11b+ adventitial cells. Targeting C3AR1 or TP receptors offers a potential therapeutic strategy in inflammatory vascular dysfunction.

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The role of phosphatidylinositol transfer proteins in maintaining sustained calcium signaling

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Keywords: Lipid-transfer, GPCR-signaling, Ca2+-signaling

Calcium (Ca^{2+})-signaling is uniquely important for cellular homeostasis regulating a range of cellular processes including cell motility, muscle contractility, hormone secretion and neurotransmitter release. Ca^{2+}-signals are generated primarily by phospholipase C β (PLC β) activating G-protein coupled receptors (GPCRs). PLCβ activation liberates inositol 1,4,5-trisphosphate (IP3) by cleaving phosphatidylinositol (PI) 4,5-bisphosphate (PIP2) in the plasma membrane (PM). IP3 then activates its Ca^{2+} channel receptors in the endoplasmic reticulum (ER) to release Ca^{2+} from the ER lumen. During GPCR activation, sustained signaling requires the maintenance of PIP2 levels by sequential PI phosphorylation by 4 kinase A (PI4KA) and PI4P 5-kinases in the PM. Since PI synthesis takes place in the ER, PI must be delivered to the PM for PIP2 generation, a process that has been attributed to class I and Class II PI-transport proteins (PITPNA/B and Nir2/3, respectively).

In the present work we used a recently identified Class I PITP inhibitor, VT01454 to investigate the contribution of Class I PITPs to maintain PM PIP2 levels and support Ca2+-signaling during stimulation of muscarinic (M1R) receptors. We followed both IP3 and Ca^{2+}-changes in HEK293 cells using transiently expressed BRET-based biosensors previously developed in our laboratory. ANOVA was used to assess statistical significance among the different groups. We found that VT01454 pretreatment caused a strong inhibition in both IP3 and Ca^{2+} signals after M1R stimulation making their increases transient, which contrasted the sustained elevations observed in M1R stimulated DMSO-treated control cells. While both IP3 and Ca^{2+} signals were strongly inhibited by VT01454, the inhibition was not as complete as that elicited by inhibition of PI4KA, which completely abolished the sustained phase of these intracellular signals. When the same experiments were repeated in cells in which Nir2 was knocked out by CRISPR/Cas9, the difference between Class I PITP and PI4KA inhibition disappeared, and both inhibitors completely eliminated the sustained phase of IP3 and Ca^{2+} signals.

These data suggest that both class I and Class II PITPs are important for sustained Ca^{2+} signaling during PLCβ activation, but PI-delivery by class I PITPs has a larger contribution. In future studies we will evaluate the relative contribution of the two classes of PITPs to the maintenance of PM levels the PI4P and PIP2 during M1R stimulation.

Hyperpolarization and cyclic nucleotide operated channel density in the arteries of hyperandrogenic rats; the effects of vitamin D.

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Keywords: hyperandrogenism, HCN channel, ivabradine, vasorelaxation

Polycystic ovary syndrome is a hyperandrogenic disorder affecting 8–13% of women of reproductive age and is often associated with vitamin D deficiency, also, with increased cardiometabolic risk. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels were described in various vascular segments, and their inhibition led to aortic relaxation

Fifty-two young female Wistar rats were divided into four groups: Control, Vitamin D-supplemented, Testosterone-treated, and Testosterone + Vitamin D co-treated. After eight weeks, thoracic aorta, carotid and renal arteries were harvested for wire myography and immunohistochemical analysis.

Results confirmed the vasodilatory effect of ivabradine on the thoracic aorta. A similar vasodilatory effect was proven on the two other arteries. Alterations of HCN channel density were detected in the smooth muscle layer of all examined arteries, and the density of specific HCN channels was influenced by hyperandrogenism (in the aorta: HCN1,2 and 4; renal artery: HCN1,3 and 4; carotid artery: only interaction between vitamin D and testosterone in case of HCN4;) or as a function of vitamin D supplementation (in the aorta: HCN1-3; renal artery: HCN 1 and 3; carotid artery: HCN3.) Vitamin D supplementation led to an enhanced vasodilatory effect of ivabradine only in the aortic rings.

These findings suggest that hyperandrogenism and vitamin D levels have distinct, region-specific effects on HCN channel expression and vascular function in PCOS.

The effect of PACAP on testicular peritubular myoid cells

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Keywords: PACAP, myoid cells, testis

The neurohormone pituitary adenylate cyclase-activating polypeptide (PACAP) has been reported to exert a variety of effects at several levels of the hypothalamic-pituitary-gonadal (HPG) axis. We have previously shown that the number of GnRH and Kisspeptin neurons in the hypothalamus is changed and the hypothalamic androgen-estrogen balance is altered in global PACAP knock-out male mice. Peritubular myoid cells play a crucial role in the transport of immotile spermatozoa along the seminiferous tubules and in the secretion of various paracrine signaling molecules. In this study, we examined how the function of peritubular myoid cells in the testis is modulated by PACAP. We performed experiments on mouse primary cell culture enriched in peritubular myoid cells isolated from CD1 wild-type mice. Our experiments revealed that PACAP at 10 and 100 nM dose significantly increased metabolic activity. We also found that alkaline phosphatase activity was higher in PACAP treated cultures. We could not detect glial cell line-derived neurotrophic factor (GDNF) release in cell culture supernatants after 24 hours. PACAP at 10 nM concentration also evoked an intracellular calcium increase, oscillations and cell contraction, which were due to both calcium release from the internal stores and calcium influx. In addition, PACAP activated the ERK/MAPK and the cAMP/PKA/CREB but not the PI3K/Akt intracellular signaling pathway. Our study demonstrates for the first time that PACAP may directly modulate the function of peritubular myoid cells by enhancing metabolic activity and inducing contraction via ERK/MAPK, cAMP/PKA/CREB pathways and intracellular calcium signaling. These findings suggest that PACAP may contribute to the regulation of seminiferous tubule dynamics and spermatogenesis at the local testicular level. Further in vivo studies are needed to clarify the physiological relevance of these results.

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Treadmill training delays aging-associated suppression in the central anorexigenic effects of leptin, alpha-melanocyte stimulating hormone and urocortin 2

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Keywords: middle-aged obesity, treadmill training, leptin, alpha-melanocyte stimulating factor, urocortin 2

Introduction: Worldwide, age-related obesity represents a growing public health burden. The efficacy of body weight–reducing mediators changes with aging, and these changes may contribute to age-dependent obesity. Our previous findings suggest that the reduced or absent central anorexigenic effects of leptin, alpha-melanocyte-stimulating hormone (alpha-MSH), and urocortin 2 (Ucn2) observed in middle-aged subjects may play a role in the development of age-related obesity. Lifestyle interventions, such as caloric restriction or increased physical activity can reduce body weight and favorably influence body composition. Previous observations also suggest that caloric restriction and increased physical activity may affect age-related alterations in the regulation of energy homeostasis.

Methods: We aimed to study the effects of a 12-week treadmill training on the central anorexigenic responsiveness to leptin, alpha-MSH and Ucn2 in middle-aged 12-month old male Wistar rats. Treadmill training started at the age of 9 months. After the 12-week training period, central anorexigenic responses were tested in an automated FeedScale system using intracerebroventricular (ICV) injections of leptin, alpha-MSH, and Ucn2. (BA02/2000-23/2025)

Results: Training resulted in a modest but significant reduction in body weight, along with favorable changes in body composition (lower fat mass and higher muscle mass). The anorexia induced by leptin, alpha-MSH, and Ucn2 increased in the active, trained group, becoming statistically significant.

Conclusions: The central efficacy of the tested food intake–reducing mediators improved in the active, trained group. Our findings raise the possibility that training delays unfavorable age-related regulatory alterations in the control of energy homeostasis. *(OTKA K138452)*

Poster Session I. - Cardiovascular physiology

P-01 The slow delayed rectifier K + current is differently regulated under baseline conditions ans1d following β-adrenergic stimulation in canine ventricular cardiomyocytes

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Keywords: Slow delayed rectifier K+ current, Sympathetic activation, Protein kinase A, Calcium/calmodulin-dependent protein kinase II, Action potential voltage clamp, Canine cardiomyocyte

Sympathetic activation robustly increases the slow delayed rectifier K+ current (IKs) in the mammalian ventricular myocardium, however, exact downstream pathways involved in the β-adrenergic regulation of the current are not fully elucidated yet. This study examined the Ca2+ sensitivity of IKs and the contribution of the protein kinase A (PKA) and the calcium/calmodulin kinase II (CaMKII) pathways in regulating IKs in isolated canine ventricular myocytes. Experiments were carried out under ß-adrenergic receptor activation (10 nM isoproterenol) and under baseline conditions (without isoproterenol). IKs was measured as an HMR-1556 sensitive current with the action potential voltage clamp technique under the physiological intracellular calcium homeostasis of the cells. Reducing intracellular Ca2+ concentration ([Ca2+]i) with 1 μM nisoldipine decreased the peak and mid-plateau densities of IKs, reduced the current integral, and increased the time-to-peak value. In contrast, ß-adrenergic receptor activation by isoproterenol resulted in larger IKs densities and integral, and shorter time-to-peak value. These effects of isoproterenol on IKs were significantly smaller when the CaMKII inhibitor 1 μM KN-93 was present in the cells, but the PKA inhibitor 3 μM H-89 did not exert such effect. Importantly, all effects of isoproterenol on IKs have fully developed even in the presence of 1 μM nisoldipine. Under baseline conditions the mid-plateau density of IKs, was significantly smaller in the presence of KN-93, H-89 or nisoldipine, while peak IKs density and the current integral were significantly smaller only in nisoldipine. In conclusion, many different signaling pathways are involved in regulating IKs. Under baseline conditions the regulation is strongly [Ca2+]i-dependent, with PKA and CaM-CaMKII involved, whereas during ß-adrenergic stimulation it is [Ca2+]i-independent and supposes a pivotal role of EPAC-mediated activation of CaMKII..

P-02 Sphingosine-1-Phosphate relaxes human coronary arteries via endothelial S1PR1 mediated NO release

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Keywords: Sphingosine-1-Phosphate; human coronary circulation; endothelium, ischemic heart disease; S1P1 receptor; NO synthase

Background: Sphingosine-1-phosphate (S1P) is a bioactive lysophospholipid that regulates multiple cardiovascular functions; however, its vasoactive effects in human coronary arteries remain poorly understood. This study aimed to investigate the role of S1P in human coronary vasomotor regulation and its alteration in ischemic heart disease (IHD).

Methods: Segments of the left anterior descending coronary artery were isolated from explanted hearts of patients with and without IHD and examined using wire myography. S1P-induced changes in vascular tone were assessed after precontraction, in some experiments with pharmacological inhibition of S1PR1, S1PR3, and nitric oxide synthase (NOS), as well as after endothelial denudation. Immunohistochemistry was performed on coronary sections using antibodies against S1PR1, S1PR3, and the endothelial marker CD31 to determine receptor localization.

Results: In coronary arteries from non-IHD patients, S1P induced marked vasorelaxation. Immunostaining revealed that both S1PR1 and S1PR3 were predominantly expressed in the endothelium. Functional experiments showed that S1P-induced relaxation was abolished by endothelial removal, NOS inhibition, and selective S1PR1 blockade, while S1PR3 inhibition had no significant effect, identifying endothelial S1PR1-mediated NO release as the main mechanism. Interestingly, S1P failed to induce vasorelaxation in arteries from IHD patients, whereas bradykinin-induced endothelium-dependent relaxation was preserved, suggesting a selective impairment of the S1P–S1PR1–NO pathway rather than generalized endothelial dysfunction.

Conclusion: These findings provide the first direct evidence that S1P induces vasodilation in human coronary arteries via an endothelial S1PR1–NOS-dependent mechanism, which is significantly impaired in IHD and may contribute to coronary vascular dysfunction.

P-03 Kinetics of nitrate-derived nitrous oxide formation as an early indicator of gastrointestinal microcirculatory dysfunction

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Keywords: nitrous oxide, ischaemia-reperfusion, microcirculation

Nitrous oxide (N_2O) is a recognized product of microbial denitrification. Although several microbial strains are theoretically capable of producing N_2O in the mammalian gastrointestinal tract, the physiological relevance of this process remains unclear, and its underlying mechanisms are poorly understood. The present study aimed to characterize the kinetics of nitrate-derived N_2O formation using ^{15}N-labelled nitrate and to evaluate exhaled N_2O as a potential non-invasive biomarker of gastrointestinal microcirculatory disturbances.

Anaesthetized, mechanically ventilated pigs received 450 mg ^{15}N-NO_3^- (99% enrichment) either enterally (n = 5) or intravenously (n = 5). Exhaled gas samples were collected every 30 minutes over a 6-hour period. ^{15}N-N_2O concentrations were determined using isotope ratio mass spectrometry and photoacoustic spectroscopy. In a separate experimental series, mesenteric ischemia–reperfusion was induced by 60-minute occlusion of the superior mesenteric artery followed by 5 hours of reperfusion (n = 5). In this model, ^{15}N-NO_3^- was administered intravenously during ischemia, and exhaled gas samples were collected throughout the reperfusion phase.

Following both enteral and intravenous administration, ^{15}N-N_2O concentrations increased after approximately 2.5 hours and peaked at around 4 hours. Photoacoustic measurements showed comparable kinetics. In contrast, during ischemia-reperfusion, peak ^{15}N-N_2O concentrations occurred markedly earlier, approximately 45 minutes after tracer administration, coinciding with the onset of reperfusion.

In conclusion, we demonstrate that intestinal microcirculatory alterations significantly influence the kinetics of nitrate-derived N_2O formation. The temporal profile of exhaled N_2O may therefore serve as a promising non-invasive biomarker for the detection of gastrointestinal circulatory disturbances.

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YP-01 Effect of laser-induced heat provocation test on gingival microcirculation in healthy subjects

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Introduction: The vasodilatory capacity (VDC) of the oral microcirculation is diminished in certain pathological conditions, such as periodontitis, diabetes, smoking, and medication-related osteonecrosis of the jaw. Reduced VDC has been linked to poorer survival rates of mucosal flaps, making its assessment a critical component of surgical planning. While changes in VDC can be effectively monitored through thermal provocation tests, traditional methods are unsuitable for the oral cavity due to its unique anatomical and spatial characteristics.

Our goal was to develop a standardized intraoral thermal provocation test and establish normative values stratified by sex, enabling the identification of patients at risk for pathological conditions.

Materials and Methods: Thermal provocation test was performed using a 0.4 W diode laser applied for 4 seconds to the marginal gingiva of tooth 12 (FDI notation) in 13 healthy, non-smoking volunteers (7 males and 6 females) with good oral hygiene. Changes in the microcirculation of the marginal gingiva were monitored using Laser Speckle Contrast Imager (LSCI).

Data were recorded during a 2-minute baseline period, throughout the heat provocation, and for 15 minutes afterward. Statistical significance was assessed using Student's t-test**.**

Results: Our results indicate that irradiation with a 0.4 W laser for 4 seconds above tooth 12 induced significant hyperemia (males: baseline LSPU = 193±46 vs. peak LSPU=287±70, p=0.003; females: baseline LSPU=237±83 vs. peak LSPU=299±75, p=0.012). A paired Student's t-test showed no difference in peak hyperemia between sexes (t=1.21, p=0.25) The peak hyperemia developed in approximately 27 seconds in both groups.

Conclusion: A laser-based, standardized intraoral thermal provocation test was successfully developed as a simple, reversible, and non-invasive method to assess the vasodilatory capacity of the gingival microvasculature. However, due to the limited number of study participants, we have not yet been able to evaluate potential gender differences.

YP-02 The role of Tenascin-C and cardiac lymphatics in left ventricular hypertrophy: insights from a mouse model

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Left ventricular hypertrophy (LVH) induced by chronic pressure overload, along with the associated risk of heart failure, can be partially mitigated by pharmacological therapies; however, its reversal remains challenging. Region-specific alterations in cardiac lymphatics may contribute to adverse tissue remodeling in LVH, yet their spatial and temporal regulation remains poorly defined, particularly in light of emerging evidence that the extracellular matrix protein Tenascin-C (TNC) regulates lymphangiogenesis.

We aimed to investigate the spatiotemporal alterations of cardiac lymphatics, in addition to examining the role of TNC and lymphangiogenesis in tissue remodeling during pressure overload-induced LVH.

LVH was induced in adult male wild-type and TNC knockout (KO) mice by transverse aortic constriction (TAC), while a subset of wild-type animals underwent sham surgery as controls. Animals were sacrificed at early (1 week) and late time points (6 weeks) after TAC, and paraffin-embedded cardiac sections were prepared. Region-specific analyses were performed using Masson's trichrome and hematoxylin–eosin staining to assess tissue morphology. Lymphatics in arterial perivascular regions, as well as TNC expression, were evaluated by fluorescent immunohistochemistry. In vitro, human lymphatic endothelial cells (LECs) were treated with human recombinant TNC for 24 hours, followed by viability assays.

Histological analyses revealed marked, region-specific alterations in lymphatic distribution in the heart under chronic pressure overload compared to sham-operated controls. Spatial and temporal analyses demonstrated that lymphatic remodeling is highly region-dependent. In wild-type mice, pressure overload reduced lymphatic density in arterial perivascular regions, concomitant with increased TNC expression. In contrast, TNC deficiency was associated with an increased number and perimeter of lymphatic vessels, along with the mitigation of adverse cardiac remodeling. In vitro findings further indicated that TNC dose-dependently negatively affects LEC viability and promotes pro-inflammatory and fibrotic endothelial–mesenchymal transition.

These results suggest that pressure overload reduces periarterial cardiac lymphatics, potentially mediated by TNC. TNC may therefore act as a regulator of lymphangiogenesis and cardiac remodeling, representing a potential therapeutic target to attenuate disease progression. Further studies are required to clarify the underlying mechanisms.

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YP-03 Characterization of the Cerebral No-Reflow phenomenon in a mouse model

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Introduction: Ischemic stroke is one of the leading causes of death worldwide. Despite the breakthrough with thrombectomy in its treatment, two-third of patients undergoing the procedure suffer from long-lasting health impairment. A primary reason for this is that successful recanalization performed within the time window fails to restore the perfusion at the microcirculatory level in nearly 30% of the cases (the no-reflow phenomenon).

Objectives: Our work aimed to characterize an experimental animal model to investigate the cellular and molecular pathomechanisms of the no-reflow phenomenon.

Methods: Measurements were performed on 3–4 month-old male mice on a C57Bl6/N genetic background under isoflurane anesthesia. The cortical cerebral blood flow (CoBF) was monitored using the laser speckle technique. In both hemispheres, we distinguished a fronto-parietal (FP) region, a temporal (T) region and, between these two, a pial-collateral (PIAL) region. Cerebral ischemia was induced by bilateral common carotid artery occlusion (BCAO) for 5 or 10 minutes, followed by reperfusion upon release of the vessels. The blood flow values were normalized to the CoBF recorded prior to the BCAO.

Results: Immediately following the BCAO, the CoBF decreased to 17.3 ± 5.8% of the baseline value. Interestingly, in 18 of 20 cases, a further marked decrease in blood flow was observed after a few minutes, resulting in severe oligemia (CoBF = 10.5 ± 3.2%), which may have been triggered by spreading depolarization (SD) as reported in the literature. During the reperfusion, the blood flow was restored more rapidly (CoBF > 75%) in mice subjected to the 5-minute BCAO than in those subjected to the 10-minute BCAO (2.0 ± 0.7 min vs. 2.9 ± 1.0 min, $p < 0.0001$). A secondary hypoperfusion (CoBF = 49.7 ± 9.4%) developed 17.3 ± 4.3 minutes after the 10-minute BCAO, and significantly earlier, 12.7 ± 2.3 minutes after the 5-minute BCAO, appearing first in the FP region. The onset of this secondary hypoperfusion correlated with the time spent in the severe oligemia (Pearson corr. coef.: 0.37; $p<0.01$), and its dynamics was significantly slower in the 10-minute occlusion group (9.2 ± 7.0 min vs. 5.5 ± 2.6 min, $p<0.01$).

Conclusions: According to our results, the applied mouse model may be suitable for studying the no-reflow phenomenon, as a secondary, lasting hypoperfusion develops following the reperfusion. This process, characterized by the dynamics depending on the duration of the severe oligemia and the specific region, may play a role in the secondary damage of the brain.

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Poster Session I. - Cellular physiology

P-04 Quinapril restores impaired autophagy in diabetic myocardium

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Keywords: diabetic myocardium, autophagy, quinapril

Autophagy is essential for maintaining cardiomyocyte homeostasis by ensuring the removal of damaged proteins and organelles. Chronic hyperglycemia disrupts autophagic flux and contributes to mitochondrial dysfunction, oxidative stress, and structural remodeling in diabetic cardiomyopathy. Although EGR-1 has previously been implicated in the regulation of autophagy and cellular stress responses, its role in diabetic myocardial autophagy is not well characterized. Furthermore, alterations in extracellular matrix (ECM) turnover—reflected by changes in MMP/TIMP balance—also contribute to disease progression. The impact of ACE inhibition on these interconnected pathways remains unclear.

Our aim was to determine whether the ACE inhibitor quinapril influences myocardial autophagic flux in type-1 diabetes and to assess its effects on EGR-1 expression and ECM regulatory components, including TIMP-1 and TIMP-2.

Type-1 diabetes was induced in 8-week-old male Sprague–Dawley rats with a single i.p. dose of streptozotocin (60 mg/kg). Animals were assigned to control, diabetic (DM), and quinapril-treated diabetic (DM+Q) groups. Quinapril was administered in drinking water (50 mg/kg/day). After 8 weeks, myocardial autophagy was assessed by Lc3-II/I ratio, p62 accumulation with immunoblot and immunofluorescence. Myocardial *Egr-1*, *Timp-1* and *Timp-2* mRNA expression was quantified. Myocardial histology was evaluated to assess structural alterations. For statistical evaluation, ANOVA with Holm-Sidak post-hoc test was performed.

Diabetes led to impaired autophagic degradation, demonstrated by increased p62 accumulation and dysregulated Lc3-II/I ratio ($p<0.05$). Quinapril significantly enhanced autophagic flux, increasing Lc3-II/I ratio while reducing p62 levels ($p<0.05$) and eliminating p62-positive aggregates as observed by immunohistochemistry. Myocardial *Egr-1* expression was elevated in non-treated diabetic rats, consistent with its known involvement in autophagy regulation, and quinapril markedly suppressed this upregulation. The increased myocardial *Timp-1* and *Timp-2* expression in non-treated diabetic rats indicates a disrupted matrix remodeling; however, quinapril treatment restored them to control levels.

In summary, quinapril effectively restored defective autophagy in diabetic myocardium and attenuated EGR-1 as well as the expression of TIMPs, mitigating ECM dysregulation. Our findings suggest that impaired autophagic flux contributes to diabetic cardiac remodeling and highlight an additional beneficial effect of ACE inhibition to attenuate myocardial injury.

P-05 Gold nanoparticles alter mitochondrial function in hypothalamic POMC neurons: potential consequences for energy balance

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Background: Gold nanoparticles (AuNPs) possess several advantageous characteristics that support their use as intracellular drug delivery systems, and interest in their diagnostic and therapeutic utility continues to expand. Their ability to enter the central nervous system (CNS) through the blood–brain barrier (BBB) depends largely on particle size and morphology. Once in the CNS, AuNPs may produce a range of effects on neuronal and glial cells, from beneficial actions that support cellular function to harmful effects that induce toxicity. The hypothalamus (HT) is likely among the earliest brain regions to encounter nanoparticles, as this neuroendocrine structure is highly exposed to circulating systemic signals because of its anatomical position and physiological role. Among the hypothalamic cell populations influenced by such factors are pro-opiomelanocortin (POMC) neurons, which are essential for metabolic regulation and homeostatic control. Mitochondrial activity in these neurons plays a central role in shaping their responses to environmental stimuli, including exposure to AuNPs, highlighting a complex interaction between nanoparticle effects and cellular metabolism in this critical brain area.

Aims: This study aimed to determine how different concentrations of AuNPs and different exposure durations influence mitochondrial activity in POMC neurons under in vitro conditions, with the goal of improving understanding of the underlying mechanisms in the hypothalamus.

Methods: The effects of multiple concentrations of gold nanoparticles on mitochondrial function in POMC neurons were examined after 1, 15, 24, and 48 h of treatment. Mitochondrial activity was analyzed using the Seahorse XFp Analyzer, allowing high-resolution metabolic assessment. In addition, mitochondrial function was further evaluated by measuring reactive oxygen species (ROS) production and cell viability.

Results: The results showed that the impact of AuNPs on mitochondrial activity is strongly dependent on both concentration and duration of exposure. In particular, AuNP treatment stimulated early cellular response mechanisms, enhanced citric acid cycle activity, and increased proton efflux, which ultimately led to impaired mitochondrial performance and reduced ATP production in POMC neurons. These alterations may disrupt hypothalamic control and energy metabolism.

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P-06 Purinergic signaling: An emerging regulator of human sebocyte biology

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Keywords: acne, $P2Y_2$ receptor, purinergic signaling, sebocyte

We have previously show that A_1, A_{2A} and A_{2B} adenosine receptors are expressed in human sebocytes, where they differentially influence sebaceous lipogenesis and immune phenotype. These data argued that purinergic signaling may contribute to the regulation of human sebocyte biology. Thus, within the confines of the current study, we aimed to further explore the role of purinergic signaling using SZ95 sebocytes.

Because the P2X purinergic receptors are dominantly Ca^{2+}-permeable ligand-gated ion channels, and many of the metabotropic P2Y receptors are coupled to G_q proteins, we first challenged the cells by an acute ATP-treatment, and found that ATP increased the $[Ca^{2+}]_{IC}$ of the sebocytes (Fluo-4 AM-based fluorescent Ca^{2+}-measurement). Importantly, the effect decreased, but did not disappear in nominally Ca^{2+}-free medium, arguing that it developed in response to the activation of ionotropic P2X and G_q protein-coupled P2Y receptors. In order to explore the expression of such receptors, we re-analyzed our unpublished RNA-Seq data, and found that multiple P2X and P2Y receptors were expressed in human sebocytes. Among these molecules, the G_q protein-coupled metabotropic $P2Y_2$ receptor exhibited a remarkably high expression level (mean RPKM value of three independent cultures: 935); hence, we decided to further investigate the expression and role of this receptor. First, we confirmed its expression using independent samples (Fast Universal TaqMan qPCR). Next, we assessed the biological effects of selective $P2Y_2$ modulators. We found that, while the $P2Y_2$ agonist PSB 1114 had no significant effect on the spontaneous, homeostatic sebaceous lipogenesis (Nile Red), it significantly increased the MTT-signal intensity and slightly, but significantly decreased the arachidonic acid-induced, excessive, "acne-mimicking" lipid production, whereas the receptor selective antagonist AR-C 118925XX significantly increased the sebaceous lipogenesis over the course of 48-h treatments.

Our data indicate that human sebocytes express multiple members of the purinergic signaling, and appropriate modulation of these molecules may emerge as a valuable tool in the management of sebaceous gland-associated diseases, e.g., acne.

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P-07 Interactions between CGRP and PACAP in the trigeminovascular system

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Keywords: CGRP, PACAP, trigeminovascular system

Migraine is a highly disabling neurological disorder predominantly affecting women. Although a therapeutic approach targeting the main sensory neuropeptide in the trigeminovascular system, the calcitonin gene-related peptide (CGRP), is effective for both relieving attacks and for preventive purposes, it is not a solution for all patients. There is an increasing experimental evidence that the release of pituitary adenylate cyclase-activating peptide (PACAP) from neurons innervating the meningeal tissues may also play a role in the pathophysiology of migraine headache, likely in cooperation with CGRP. To clarify this, we studied the release of CGRP and PACAP in the dura mater in response to a nociceptive stimulus. We also investigated interactions between the two neuropeptides that may be relevant to the pathophysiology of migraine.

The amount of CGRP and PACAP released from meningeal nociceptors was measured in ex vivo dura mater preparations of male and female Wistar rats after application of the transient receptor potential vanilloid 1 receptor agonist capsaicin. Modifying effect of PACAP on the CGRP release was also measured. Effects of the two neuropeptides and their interactions on the meningeal blood flow was measured in an open cranial window model with laser Doppler flowmetry. The distribution of CGRP- and PACAP-immunoreactive meningeal nerve fibres was also studied in whole mount preparations of the dura mater.

We found gender differences in both basal and stimulated peptide release from meningeal afferents. A striking difference was that females responded to capsaicin stimulation with a much higher peptide release than males. Pretreatment of the dura mater with PACAP modified both the CGRP release and the CGRP-induced increase in meningeal blood flow.

Our results indicate that complex interactions may occur between the two peptides when they are released in meningeal tissues, a fact that must be taken into account in the therapeutic management of migraine headaches.

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P-08 The effect of genetic ablation of PACAP on testicular structure

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Keywords: PACAP, testis, Sertoli cells

The function of hypothalamus-pituitary-gonadal (HPG) axis is modulated at multiple levels by the neurohormone pituitary adenylate cyclase-activating polypeptide (PACAP). We have previously demonstrated hypothalamic alterations in global PACAP knock-out male (KO) mice including changes in the number of GnRH and Kisspeptin neurons as well as altered hypothalamic androgen-estrogen balance. While the effects of PACAP on fertility are well characterized in females, considerably less data are available for males.

The aim of our study was to investigate potential structural and expression changes in the testes of PACAP KO mice that may explain the observed fertility impairments.

We performed experiments on 3- and 7-month-old wild-type and global PACAP KO male mice. RNAscope in situ hybridization technique and immunofluorescence stainings were carried out on whole-mount preparation of seminiferous tubules and cryosections.

Our measurements revealed that the testes of 3-month-old PACAP KO animals were smaller, which could be attributed to a reduction in the interstitial compartment. However, serum testosterone levels were comparable between the two groups, and no differences were observed in the expression levels of the four key enzymes involved in testosterone synthesis. In contrast, the number of Sertoli cells that are essential for gametogenesis and testicular estrogen production significantly increased in both 3- and 7-month-old PACAP KO animals. The number of GFRα1-positive spermatogonia remained unchanged. The interstitial autofluorescence was significantly reduced in PACAP KO animals, suggesting delayed testicular ageing. Finally, lipid staining with Nile Red revealed lower apolar lipid content in the interstitium of PACAP KO males.

In conclusion, PACAP deficiency induces significant structural and cellular alterations in the testis without affecting circulating testosterone levels, indicating the presence of compensatory mechanisms. Further studies are needed to elucidate the underlying pathways responsible for the observed structural changes in PACAP KO animals.

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P-09 The role of TRPA1 in Pancreatic Injury and Fibrosis

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Introduction Transient Receptor Potential Ankyrin 1 (TRPA1), a chemosensitive ion channel involved in neurogenic inflammation and pancreatic pain signaling, has been implicated in pancreatitis-related nociception, yet its role in pancreatic injury and fibrotic remodeling across acute (AP) and chronic pancreatitis (CP) remains poorly defined. This study investigates TRPA1's role in distinct murine models of AP and CP.

Methods: Wild-type and TRPA1-knockout C57BL/6 mice were used. AP was induced by intraperitoneal (i.p.) hourly injections of cerulein (10×50μg/kg) or ethanol/palmitoleic acid (2×1.35g/kg and 2×150mg/kg); CP was induced by repeated cerulein injections (8×50μg/kg i.p.) every third day for two weeks. Pancreatic tissues and blood were collected. Pancreatic injury was assessed via edema, leukocyte infiltration, acinar cell damage, and serum amylase, while fibrosis was evaluated by Picrosirius Red, vimentin, and glial fibrillary acidic protein (GFAP) staining.

Results In both AP models, TRPA1-knockout did not significantly alter key markers of acute pancreatic injury, including edema, leukocyte infiltration, acinar cell damage, or serum amylase levels, compared with wild-type controls. In CP model, Picrosirius Red staining revealed no significant difference in collagen fiber deposition between wild-type and TRPA1-knockout mice. However, TRPA1-knockout mice showed significantly attenuated expression of Vimentin and GFAP over the two-week induction period compared to wild-type mice which exhibited progressive fibrotic remodeling.

Conclusion: TRPA1 may have a negligible influence in early pancreatic injury in AP, yet drive fibrotic progression in CP. These findings might position TRPA1 as a selective mediator of chronic pancreatic remodeling and a potential therapeutic target in CP.

P-10 Investigating biased signaling of the AT_1 angiotensin receptor using conformational- and activation-based biosensors

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Introduction: The angiotensin II type 1 receptor (AT1R) is a key regulator of blood pressure and fluid homeostasis, and it is a major therapeutic target for hypertension and chronic heart failure. Recent research has focused on biased signaling – where ligands selectively activate certain pathways over others – as such agonists may offer therapeutic benefits. However, the mechanism of biased signaling of AT1R remains incompletely understood.

Methods: We have developed a conformation-sensitive nanobody (Nb.AT110i1)-based biosensor. This sensor and a panel of other sensors (mini G protein biosensors, G protein activation and β-arrestin recruitment sensors, and sensors for phosphatidylinositol 4,5-bisphosphate depletion and inositol 1,4,5-trisphosphate production) were transiently transfected in HEK 293T cells. Various AT1R agonists were tested, with angiotensin II (AngII) serving as a reference ligand.

Results: TRV120027, a β-arrestin biased peptide agonist, evoked a several-fold larger BRET ratio increase than AngII for Nb.AT110i1, while both ligands produced comparable BRET ratio increases for β-arrestin2. Furthermore, the small-molecule agonist L-162,313 did not show detectable response when measured with the TRUPATH Gq sensor. Bias signaling analysis showed that TRV120027 is a better activator of the Nb.AT110i1 sensor than AngII, and L-162,313 has a bias profile closely resembling that of a β-arrestin biased ligand.

Conclusion: These findings indicate that Nb.AT110i1 selectively recognizes a receptor conformation enriched by β-arrestin-biased ligands. Such states appear capable of supporting β-arrestin engagement without fully adopting the Gq-activating conformation. Our findings suggest that L-162,313 induces a signaling profile characterized by low Gq/11 activation and maintained β-arrestin recruitment. Hence L-162,313 should be classified as a β-arrestin–biased AT1R agonist with a hitherto unknown mechanism, and may serve as a lead compound for the development of novel non-peptide biased ligands.

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P-11 Investigation of the mechanism of ferroptosis in melanoma

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Keywords: ferroptosis, melanoma, pigmentation, dermatology

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P-12 Application of modified GRP1-PH domains as biosensors of various cellular processes

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Keywords: phosphatidylinositol 3,4,5-trisphosphate; pleckstrin homology domain; bioluminescence resonance energy transfer

Phosphoinositide-binding pleckstrin homology (PH) domains interact with both phospholipids and proteins, often complicating their use as specific lipid biosensors. In this study, we introduced specific mutations (K340L and I307E) into the phosphatidylinositol 3,4,5-trisphosphate (PIP3)-specific PH domains of protein kinase B (Akt) and general receptor for phosphoinositides 1 (GRP1) that disrupt protein-mediated interactions while preserving lipid binding, in order to enhance biosensor specificity for PIP3, and evaluated their impact on plasma membrane (PM) localization and lipid-tracking ability. Using bioluminescence resonance energy transfer (BRET) we assessed the localization of PH domains in HEK293A cells under different conditions. While Akt-PH mutants showed minimal deviations from the wild type, GRP1-PH mutants exhibited significantly reduced PM localization both at baseline and after stimulation with epidermal growth factor (EGF), insulin, or vanadate. We further developed tandem mutant GRP1-PH domain constructs to enhance PM PIP3 avidity. Additionally, our investigation into the influence of ADP ribosylation factor 6 (Arf6) activity on GRP1-PH-based biosensors revealed that while the wild-type sensors were Arf6- dependent, the mutants operated independently of Arf6 activity level. These optimized GRP1-PH constructs provide a refined biosensor system for accurate and selective detection of dynamic PIP3 signalling, expanding the toolkit for dissecting phosphoinositide-mediated pathways. In addition, using confocal microscopy we also noticed a surprising cell division dependent translocation of these PH domains allowing their application as biosensor of this process.

P-13 Uncoupling ER Redox networks – the possible absence of the thioredoxin system

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Keywords: endoplasmic reticulum, thioredoxin reductase

Introduction: The endoplasmic reticulum (ER) lumen is generally considered to be an oxidative environment, as it serves as the primary site of protein thiol oxidation during their post-translational modification, although reducing processes requiring NAD(P)H are also found here. In most cellular compartments, the thiol/disulfide and reduced/oxidized pyridine nucleotide systems are enzymatically linked to form complex redox networks. However, the parallel presence of oxidized thiol-disulfides and reduced pyridine nucleotides suggests that the ER lumen may lack elements that link these two systems together.

Aims: We wanted to confirm the luminal deficiency of one of these coupling systems, the thioredoxin (Trx) / thioredoxin reductase (TrxR) proteins, and to examine the effects of their artificial expression.

Methods: We carried out an in silico analysis to predict the localization of each Trx/TrxR isoform. We measured TrxR activity using a colorimetric assay in various organelles. The protein expression of each isoform in subcellular fractions was examined by Western blot analysis. Their intracellular distribution was further studied by immunofluorescence microscopy. The cell viability of transiently transfected cells expressing Trx1 and TrxR1 in the ER lumen was measured, and the induction of apoptosis was examined by Western blot.

Results: *In silico* predictions showed a uniformly very low probability of luminal localization for each isoform (0–5%). Analysis of rat liver subcellular fractions revealed that none of the Trx/TrxR isoforms are expressed in the ER. Immunofluorescence analysis confirmed that Trx and TrxR isoforms did not colocalize with the ER-marker Grp94. The specific activity of TrxR in the ER was nearly zero (0,02 U/mg ± 0,01), while we measured higher activities in the cytoplasm (1,26 U/mg ± 0,11) and mitochondria (1,57 U/mg ± 0,19). Transient transfection of HeLa cells with ER-targeted Trx1 and TrxR1 resulted in a rapid decrease in cell viability and induction of apoptosis.

Conclusions: Our results suggest that none of the components of the Trx/TrxR system are expressed in the ER lumen. The absence of this electron transfer chain may explain the uncoupling of the luminal redox systems, allowing the parallel occurrence of a reduced pyridine nucleotide pool and oxidized proteins.

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P-14 Pretreatment with NAD+ Precursor (NMN) has cardioprotective effects in aged mouse hearts

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Keywords: NAD^+, NMN, aging, mouse, heart, cardioprotection

During aging, nicotinamide adenine dinucleotide (NAD^+) depletion and impairment of cardiac ischemia tolerance can develop. Our aim was to explore whether treatment with NAD^+ precursor (nicotinamide mononucleotide, NMN) improves heart function and prevents enhanced ischemic damage in old hearts.

After pretreatment of young and old (4 and 26 months old) C57Bl/6N male mice with NMN (500 mg/kg/day ip.) or carrier (PBS) for 2 weeks, echocardiographic examination was performed. Afterwards, hearts were isolated and perfused with constant pressure in a Langendorff system. The hearts were exposed to 23 minutes of global ischemia, followed by a 120-minute reperfusion period. Coronary flow and left ventricular (LV) pressure changes were measured, and LV infarct size was determined.

In elderly hearts, echocardiography showed enlargement of the left ventricle, however ejection fraction was preserved. NMN treatment did not affect the echocardiographic parameters. The ratio of the infarcted area in LV of old mice was significantly higher compared to the young mice. After NMN pretreatment, the myocardial infarction size of the old hearts decreased. The postischemic coronary flow and functional restitution of old hearts were impaired compared to young controls, however NMN treatment restored this impairment. Pretreatment with NAD^+ precursor does not affect the resting heart function, whereas it significantly reduces the consequences of cardiac ischemia in hearts of aged mice; it has a favorable effect both on infarct size and on postischemic functional recovery.

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P-15 Random positioning machine-induced microgravity alters the proliferative capacity of C2C12 myoblasts

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Keywords: microgravity, proliferation, C2C12, skeletal muscle

Skeletal muscle plays a pivotal role in numerous physiological functions. Proper muscle function is essential for maintaining quality of life, and muscle tissue possesses an extraordinary ability to regenerate driven by satellite cells. In response to stimuli, such as injury, dormant satellite cells are activated, enter the cell cycle, divide, differentiate, and then fuse with each other or with damaged myotubes. However, these cellular processes are strictly regulated and require remarkable cytoskeletal reorganization. Septin7 is a crucial member of the septin family of GTP-binding proteins, often referred to as the fourth element of the cytoskeleton. Our previous results verified that knocking down Septin7 in myogenic cells interrupts the ability to divide and fuse into myotubes, highlighting its crucial role in skeletal muscle regeneration. Disruptions to physical activity, for instance in elderly people, bedridden patients, or astronauts exposed to weightlessness, can significantly affect muscle strength and consequently, the regenerative capacity of muscle tissue. Therefore, it is important to investigate the effects of weightlessness induced by low gravity at the cellular level.

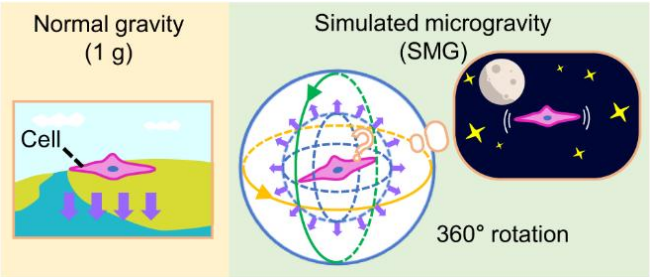

Figure 1. *Principle for generating a simulated microgravity environment using the random positioning machine*

Microgravity (MG) was simulated with a random positioning machine (RPM) placed in a CO_2 incubator, and the alterations in cell number, viability, proliferation, and cell cycle were monitored. Our experiments suggest that microgravity has a negative effect on the proliferation of myoblasts. Following incubation in the RPM device, the number of cells decreased; this was not due to cell death, as no significant cell death was observed. However, a fluorescence-based proliferation assay confirmed that, under microgravity conditions, the cells' ability to divide was remarkably lower than the non-rotating group. Our results also revealed that MG interferes with the late phase of the cell cycle, as a significant number of cells accumulated in the G2/M phase. Based on immunocytochemical images, it can be concluded that the filamentous structure of Septin7 fragments under MG, and this process is accompanied by an altered cell morphology, as the elongated shape changed to more rounded one. Overall, our results suggest that *in vitro* simulated weightlessness exerts a negative effect even during the early stages of muscle regeneration, which also influences structural changes in the septin filaments.

P-16 GPX8 deficiency drives metabolic vulnerability via INSIG1-dependent lipid dysregulation

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Background: Glutathione peroxidase 8 (GPX8) is an endoplasmic reticulum-resident redox enzyme implicated in the regulation of cellular stress responses. Emerging evidence suggests that GPX8 may also participate in lipid metabolism, calcium homeostasis, and autophagy. However, its physiological role in metabolic adaptation and lipid homeostasis remains poorly understood.

Aims: This study aimed to investigate the role of GPX8 in lipid metabolism and cellular stress adaptation, with particular focus on adipogenic differentiation, lipid droplet formation and transcriptional regulation associated with insulin-induced gene 1 (INSIG1).

Methods: GPX8 knockout mouse embryonic fibroblasts (MEFs) were generated using CRISPR-Cas9 technology. Cellular viability was assessed following treatment with palmitic acid and stearoyl-CoA desaturase 1 (SCD1) inhibitors. Lipid droplet formation and adipogenic differentiation were analyzed by BODIPY 493/503 staining. Gene expression changes were determined by RT-qPCR. In addition, a genome-wide CRISPR-Cas9 knockout screen was performed to identify genes whose loss rescues GPX8-deficient cells from lipotoxic stress.

Results: GPX8-deficient MEFs displayed markedly increased sensitivity to lipotoxic cell death induced by palmitic acid and SCD1 inhibition. During adipogenic induction, GPX8 knockout cells showed impaired expression of key lipogenic genes including SCD1, fatty acid synthase (FASN) and peroxisome proliferator-activating receptor gamma (PPARγ). Elevated INSIG1 expression was observed in GPX8-deficient cells, suggesting suppression of sterol regulatory element-binding protein 1c (SREBP1c) activation and reduced lipogenic capacity. Consistent with this, the genome-wide CRISPR screen identified INSIG1 as one of the top candidates whose deletion restored viability under lipotoxic conditions. GPX8-deficient cells also exhibited increased glycolytic activity and lactate secretion, indicating broader metabolic rewiring.

Conclusion: GPX8 is an important regulator of lipid homeostasis and metabolic stress resistance. Loss of GPX8 sensitizes cells to saturated fatty acid overload and impaired desaturation capacity, likely through INSIG1-dependent suppression of lipogenic adaptation. These findings identify GPX8 as a key determinant of cellular metabolic flexibility under lipid stress.

P-17 Genetically modified HoxB8 progenitor cells for functional analysis of osteoclastogenesis

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Background: Osteoclasts are multinuclear giant cells originating from the myeloid lineage of the hematopoietic system. As the only cell type capable of bone resorption, they play a central role in maintaining skeletal homeostasis. Impaired osteoclast formation or function causes osteopetrosis, whereas excessive bone resorptive activity occurs during osteoporosis, inflammatory bone erosion, and osteolytic bone metastases. Due to their role in physiological and pathological bone metabolism, understanding the molecular and cellular mechanisms that control osteoclast biology remains a significant research priority.

Methods: Our research group has previously established a HoxB8-based system in which mouse bone marrow cells were retrovirally transduced with ER-HoxB8 fusion protein. In the presence of β-estradiol, the HoxB8 is activated, resulting in the generation of conditionally immortalized HoxB8 progenitor cells. Upon β-estradiol withdrawal, these progenitors can be differentiated toward multiple myeloid cell types.

Aims: Our aim is to develop a HoxB8 cell line that can be differentiated toward osteoclast-like cells and to apply the lentiviral CRISPR/Cas9 system for targeted gene deletions in these progenitors.

Results: Upon stimulation with M-CSF and RANKL for six days, the HoxB8 progenitors differentiated toward multinucleated, TRAP-positive cells, confirming the osteoclastogenic potential of the system. We are currently performing CRISPR/Cas9-based gene editing to generate HoxB8 cell lines that lack RANK or DC-STAMP, supposedly leading to defective differentiation into osteoclasts and fusion into multinucleated cells, respectively.

Conclusion: Our results demonstrate that the HoxB8 system is an efficient method for functional studies of osteoclast differentiation and activity.

P-18 Platelet SYK promotes solid tumor metastasis via TXA_2-mediated immunosuppression

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Keywords: platelet, solid tumor cell, metastasis, spleen tyrosine kinase, thromboxane A2

Metastasis remains the leading cause of cancer-related mortality and involves complex interactions between tumor cells and host components. Among these, platelets have emerged as critical facilitators of metastatic dissemination, yet the molecular mechanisms underlying platelet activation in this context are incompletely understood. This study investigates the role of spleen tyrosine kinase (SYK), a key signaling molecule in platelet activation, in promoting solid tumor metastasis and tumor-associated immunosuppression.

Using both genetic and pharmacological approaches, we demonstrate that platelet-specific deletion of SYK significantly reduces metastatic burden in murine models of melanoma and colorectal carcinoma. Importantly, inhibition of SYK does not affect primary tumor growth, indicating a specific role in metastatic progression. Pharmacological inhibition with clinically relevant SYK inhibitors similarly decreases metastasis formation, supporting the translational relevance of these findings.

Mechanistically, we identify thromboxane A_2 (TXA_2) as a critical downstream effector of platelet SYK signaling. SYK-expressing platelets produce elevated levels of TXA_2, whereas SYK-deficient platelets show markedly reduced TXA_2 synthesis. Functional studies reveal that platelet-derived TXA_2 suppresses the proliferation and effector functions of $CD8^+$ cytotoxic T cells, thereby impairing anti-tumor immunity. Restoration of TXA_2 signaling rescues metastatic potential even in SYK-deficient conditions, highlighting its central role in this pathway.

Further, pharmacological blockade of TXA_2 production phenocopies the effects of SYK inhibition, leading to reduced metastatic spread and enhanced $CD8^+$ T cell responses. Combined inhibition strategies do not yield additive effects, suggesting that SYK and TXA_2 act within the same signaling axis. Importantly, depletion of $CD8^+$ T cells abolishes the protective effect of SYK deficiency, confirming that the anti-metastatic benefit is mediated through immune modulation.

Collectively, these findings establish platelet SYK as a critical regulator of tumor metastasis through a TXA_2-dependent immunosuppressive mechanism. By linking platelet activation to suppression of cytotoxic T cell function, this study uncovers a novel pathway by which tumors evade immune surveillance during dissemination. Given that several SYK inhibitors are already in clinical development, targeting the platelet SYK–TXA_2 axis represents a promising therapeutic strategy to enhance anti-tumor immunity and prevent metastatic disease.

P-19 The effect of hyaluronidase treatment on adipogenesis of orbital fibroblasts

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Hyaluronan (HA) overproduction and enhanced *de novo* adipocyte differentiation of orbital fibroblasts (OFs) are characteristics of thyroid eye disease (TED). Adipogen stimuli further exacerbate HA accumulation. The role of HA in the adipogenesis varies by the origin of the cells. The aim of our study was to examine how HA deprivation by external hyaluronidase (HYAL) treatment affects the adipogenesis in OFs.

Adipogenic induction was used for 12 days in the presence or absence of HYAL on OFs derived from the orbital connective tissue of TED patients (n=7). HA concentration was measured by ELISA. The lipid accumulation was verified by Oil Red O (ORO) staining. The expression of the enzymes of HA metabolism, synthases (HAS1, HAS2 and HAS3) and hyaluronidases (HYAL1, HYAL2 and CEMIP), and regulators of adipogenesis: PPARγ and CEBPα, as well as the terminal marker fatty acid binding protein 4 (FABP4) were measured by RT-PCR.

TED OFs were able to differentiate into adipocytes. This was accompanied by HA accumulation, characterized by increased HAS2 ($p<0.05$) and decreased HYAL1 ($p<0.01$) and CEMIP expression ($p<0.05$). With HYAL treatment, HA disappeared from the culture media ($p<0.001$) and markedly decreased in the pericellular coat ($p<0.01$). Cell viability was not compromised by HA degradation. ORO staining confirmed that adipogenesis was diminished by HYAL treatment as verified by decreased PPARγ ($p<0.01$), CEBPα ($p<0.05$) and FABP4 ($p<0.05$) expressions.

Our findings indicate that two important factors in TED pathogenesis, HA accumulation and adipogenesis, can be modulated by HYAL treatment.

P-20 Diversity of β-arrestin binding in GPCRs: the role of core and C-terminal interactions

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Keywords: GPCR, β-arrestin, desensitization

Background: β-arrestins are involved in termination of GPCR signalling (desensitization) and internalization. They bind to receptors in two main ways: via a core interaction to the transmembrane core of the receptor, and via a C-terminal interaction to the phosphorylated intracellular tail region. GPCRs are traditionally classified into class A and B based on the stability of β-arrestin binding. Class A receptors (e.g. β2AR, V1A, CB1) show transient binding, whereas class B receptors (e.g. AT1R, V2R) form stable complexes that internalize into endosomes. The binding preferences of β-arrestin1 and β-arrestin2 isoforms may differ, but the structural basis of this is not fully understood.

Aims: Our aim was to map in detail the β-arrestin binding of different GPCRs. We investigated the role of the core and C-terminal binding interfaces of β-arrestins in numerous GPCRs, as well as how these influence the binding of β-arrestin1 and β-arrestin2 isoforms in class A and B receptors.

Methods: We examined activation differences of β-arrestin1/2 isoforms and binding-site mutants in numerous class A and B GPCRs. We performed BRET-based measurements and confocal microscopy in cells expressing wild-type and mutant β-arrestins. Two mutant β-arrestins were used: K2A, which impairs the C-terminal interaction, and ΔFLR, which impairs the core interaction.

Results: The individual mutations reduced β-arrestin activation in certain receptors as expected, but to varying degrees; however, in other cases the loss of one binding interface enhanced recruitment through the other pathway. In some receptors both interaction interfaces were required, and mutation of either binding interface abolished β-arrestin activation.

Conclusions: Our results suggest that GPCRs cannot be simply classified into class A and B based on βarrestin binding. Even receptors classified into the same class show significant differences: the contribution of core and C-terminal interactions differs and may also interact with each other, even in an inhibitory manner. The different preferences of β-arrestin1 and β-arrestin2 further increase the complexity of the system, suggesting a multi-state binding model.

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P-21 Lentivirus based genetic manipulation of neutrophil progenitor cells

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Keywords: CRISPR screening, HoxB8, neutrophil progenitors

Although several genes have already been thoroughly characterized in neutrophil function, the short lifespan of these cells makes their direct manipulation unfeasible thus posing a significant obstacle in the way of neutrophil studies at single-protein resolution.

We tried to circumvent this hurdle using so-called HoxB8 neutrophil progenitor cells. These cells maintain a proliferating/self-renewing progenitor phenotype providing ample opportunity for genetic interventions but may also be easily differentiated to functioning neutrophil granulocytes both in vitro and in vivo.

First, we generated HoxB8 cells either from primary bone marrow cells already expressing Cas9. Next, we performed a pooled CRISPR screen using the Brie sgRNA library to enable genome-wide interrogation of gene function in HoxB8 progenitor cells. The efficiency and representation of the library were first validated in vitro following lentiviral transduction, ensuring adequate coverage and uniform sgRNA distribution. Transduced progenitor cells were subsequently transplanted in vivo, where they underwent differentiation into mature neutrophils. After in vivo maturation, cells were isolated from bone marrow and spleen samples, and sgRNA representation was analyzed to identify genes influencing neutrophil development, survival, or tissue distribution.

In conclusion, lentiviral transduction of HoxB8 progenitor cells is a powerful tool for the genetic manipulation of the neutrophil compartment. The CRISPR/Cas9 system can be used to reliably create new cell lines using gene deletion or activation methods facilitating the study of neutrophil biology and potentially leading to the discovery of important new pharmacological targets or disease biomarkers.

P-22 The C-terminal domain of peroxidasin as a novel matrikine

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YP-04 Investigation of the expression and role of TRPM2 ion channel in human sebocytes

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Keywords: human skin, sebaceous gland, sebocyte, TRP channel

We have previously shown that several members of the transient receptor potential (TRP) ion channel superfamily are negative regulators of sebaceous lipogenesis. Moreover, activation of TRPV3 was found to induce an inflammatory response in human sebocytes. Importantly, according to our unpublished RNA-Seq data, another TRP channel, namely TRPM2, may also be expressed in these cells. Thus, in the current study, we aimed to investigate the expression and putative role of TRPM2 in human sebocytes.

Viability, lipid synthesis, gene expression, and mediator release of human SZ95 sebocytes were monitored using MTT-assay, Nile Red labeling, RNA-Seq, RT-qPCR, western blot, and ELISA, respectively. The cells were treated using known TRPM2-modulators (activator: GEA 3162; antagonist: ACA) or vehicle.

First, we investigated the expression of TRPM2, and confirmed its presence at both the mRNA and the protein level. Next, using the selective TRPM2 agonist GEA 3162, we found that, when applied at non-cytotoxic concentrations, it significantly elevated sebaceous lipogenesis. This effect appeared to be at least in part TRPM2-mediated, since co-administration of the TRPM2-antagonist ACA decreased it. Next, the effect of GEA 3162 on the immune phenotype of sebocytes was also investigated. We found that the TRPM2 activator GEA 3162 significantly increased the mRNA expression of interleukin (IL)-1β and IL-6 over the course of 24-h treatments, while it decreased the release of IL-8. In order to unveil the mechanism of the above effects, we performed RNA-Seq analysis following 24-h treatments using GEA 3162 or vehicle. The analysis revealed that GEA 3162 significantly (≥2 fold-change, $p<0.05$) up-regulated 332, and down-regulated 258 genes. Furthermore, phosphokinase array identified several GEA 3162-regulated signaling molecules (c-Jun, CREB, JNK 1/2/3, Lyn, and STAT6) that might be involved in mediating its effects.

Our data indicate that TRPM2-modulators may influence the biological processes of human sebocytes, and GEA 3162 may increase sebaceous lipogenesis in an at least in part TRPM2-dependent manner.

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YP-05 Cardioprotective and antiarrhythmic effects of a novel H_2S-donating ascorbic acid derivative (BM-164) in diabetic myocardium subjected to ischemia/reperfusion

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Diabetes mellitus significantly increases the susceptibility of the myocardium to ischemia/reperfusion (I/R) injury and life-threatening ventricular arrhythmias. Hydrogen sulfide (H_2S) is a potent endogenous cardioprotective mediator, yet its therapeutic potential in diabetic hearts remains underexplored.

In our work we investigated the effect of BM-164, a novel H_2S-donating ascorbic acid derivative, in isolated hearts from Zucker diabetic fatty (ZDF) rats using the Langendorff perfusion model (30 min ischemia, 120 min reperfusion).

The incidence of ventricular fibrillation (VF) after ischemia was similar in lean and diabetic control hearts; however, BM-164 pretreatment dramatically reduced VF incidence from 92% to 8%. Infarct size tended to decrease in BM-164–treated hearts compared to both controls, though not reaching statistical significance. H_2S measurements confirmed robust donor release during perfusion. At the molecular level, western blot analysis of heart samples revealed that BM-164 increased p62 levels and modulated LC-3i/II expression, indicating active regulation of autophagy, while cystathionine β-synthase (CBS) expression was reduced, suggesting negative feedback on endogenous H_2S production. These findings indicate that BM-164 not only delivers exogenous H_2S but also modulates redox balance, autophagy, and cellular stress responses, exerting antiarrhythmic and cardioprotective effects in diabetic myocardium.

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YP-06 Regulation of TREK K^+ channels by intracellular calcium

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Keywords: TREK-1, TREK-2, Calcineurin, Electrophysiology

Two-pore domain potassium (K2P) channels are background K^+ channels that play a central role in the regulation of membrane potential and cellular excitability. Among them, TREK-1 (K2P2.1) and TREK-2 (K2P10.1) are widely expressed and responsive to diverse physiological stimuli, while the related TRESK (K2P18.1) channel is classically activated by intracellular Ca^{2+} signals via the protein phosphatase calcineurin. In this study, we investigated whether TREK channels are also subject to Ca^{2+}-dependent regulation and aimed to elucidate the underlying molecular mechanisms.

Experiments were conducted in the *Xenopus laevis* oocyte expression system using two-electrode voltage clamp electrophysiology. Elevation of intracellular Ca^{2+}, induced either by extracellular application of the Ca^{2+} ionophore ionomycin or by microinjection of inositol 1,4,5-trisphosphate (IP_3), resulted in a robust activation of TREK-1 currents. This effect was abolished by intracellular Ca^{2+} buffering with EGTA, confirming the dependence on increased cytoplasmic Ca^{2+} levels. In contrast, the related channel TRAAK (K2P4.1) was not affected under the same conditions. TREK-2 exhibited low basal activity in oocytes but could be selectively activated using the pharmacological opener ML-335, which enabled the detection of a Ca^{2+}-dependent stimulatory effect.

Further pharmacological and molecular analyses demonstrated that this regulation is mediated by the Ca^{2+}/calmodulin-dependent phosphatase calcineurin. Inhibition of calcineurin with cyclosporin A or FK506 abolished ionomycin-induced activation of TREK channels. Conversely, coexpression of a constitutively active form of calcineurin enhanced TREK-1 background currents, an effect that was reversed by FK506. Mutational analysis supported this mechanism, as substitution of key phosphorylation sites in TREK-1 (S300A/S333A) eliminated the Ca^{2+}-dependent response. In addition, the scaffolding protein AKAP5 (AKAP79/150) significantly potentiated TREK-1 activation, whereas disruption of its calcineurin-binding motif reduced this effect, indicating that anchored calcineurin facilitates efficient channel regulation.

Together, these findings demonstrate that, similarly to TRESK, TREK-1 and TREK-2 are subject to Ca^{2+}-dependent regulation, although via a distinct mechanism involving AKAP5-anchored calcineurin.

YP-07 Characterization of brain calcium efflux dynamics by high-resolution fluorespirometry under anoxic conditions

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Keywords: calcium, mitochondria, fluorespirometry, brain, anoxia

Introduction: Calcium (Ca^{2+}) is a key regulator of mitochondrial function, stimulating oxidative phosphorylation at low concentrations while triggering mitochondrial permeability transition pore (mPTP) opening and cell death at higher levels. Although Ca^{2+} dynamics have been successfully examined in peripheral tissues such as the liver, their assessment in the central nervous system (CNS) has not yet been established. Our aim was to develop and optimize a method for detecting mitochondrial Ca^{2+} efflux under anoxic conditions in the brain.

Materials and Methods: Whole-brain homogenates were prepared from 20-25 g male C57BL/6N mice (n = 11) and used for all experiments. Ca^{2+} and O_2 fluxes were measured by high-resolution respirometry using a blue fluorescent sensor in the presence of 1 μM CaGreen-5N. Mitochondrial Ca^{2+} fluxes were stimulated by a hypoxia–anoxia transition within the respiratory chamber under various substrate and inhibitor conditions.

Results: The transition from hypoxia to anoxia induced an immediate increase in CaGreen-5N fluorescence, regardless of whether respiration was supported by glutamate+malate (NADH electron transfer-pathway state), rotenone+succinate (succinate pathway state), or malate+octanoylcarnitine (fatty acid oxidation pathway state) prior to oxygen depletion. The presence of a saturating concentration of ADP (2.5 mM) delayed Ca^{2+} efflux during anoxia, whereas CGP37157 (5 and 30 μM), an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger (mtNCX), had no effect on mitochondrial Ca^{2+} release. The indicator remained responsive to the addition of exogenous Ca^{2+} (8 μM) following stabilization of maximal efflux, and the fluorescence signal was completely abolished by the Ca^{2+} chelator EGTA (1 mM).

Conclusion: The hypoxia–anoxia transition elicited a rapid mitochondrial Ca^{2+} efflux in brain homogenates that was largely independent of the respiratory substrate, indicating a robust response of mitochondrial Ca^{2+} handling to oxygen deprivation. The absence of an effect of CGP37157, together with the delay induced by saturating ADP, suggests that this process is not primarily mediated by the mtNCX, but is instead linked to the bioenergetic state and possibly mPTP-associated mechanisms. This approach provides a sensitive platform to probe mitochondrial Ca^{2+} dynamics in the CNS and may guide future studies of mitochondrial dysfunction.

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YP-08 TRPV4 inhibition suppresses spreading depolarization and astrocyte reactivity in acute brain slices

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Keywords: spreading depolarization, edema, TRPV4 inhibition

Introduction: During acute ischemic stroke (AIS), ATP depletion leads to spreading depolarization (SD), astrocyte swelling, and the development of cytotoxic edema. These processes contribute to secondary tissue injury and expansion of the ischemic lesion. TRPV4 is a non-selective cation channel with an important role in astrocyte volume regulation and calcium signaling; therefore, it may represent a promising therapeutic target for attenuating edema formation and SD propagation.

Objective: The aim of our study was to investigate the effects of TRPV4 channel inhibition on tissue swelling and SD in mouse acute brain slices.

Methods: Coronal brain slices (350 µm) were prepared from C57BL/6 mice (n = 20). Edema was induced using hypoosmotic medium (HM; 130 → 60 mM NaCl), while SD was triggered by hypoxia. The spatial and temporal characteristics of SD were recorded by white-light reflectance imaging, and brain tissue edema was quantified using the wet/dry weight method. TRPV4 channels were blocked with HC067047 (HC; 10 µM). Astrocyte reactivity was assessed by GFAP immunohistochemistry, while neuronal viability was evaluated using NeuN staining.

Results: HC067047 reduced SD propagation velocity (3.8 ± 1 vs. 6.4 ± 1.9 mm/min; HM60+HC vs. HM60) and decreased the SD-affected cortical area (54.8 ± 13.4 vs. 74.5 ± 15.4%; HM60+HC vs. HM60). TRPV4 inhibition also reduced the number of GFAP-positive astrocytes (8 ± 5 vs. 18 ± 5 vs. 4 ± 1 cells/100 µm^2; HM60+HC vs. HM60 vs. naïve). In contrast, it had no significant effect on neuronal viability (6 ± 2 vs. 4 ± 1 vs. 13 ± 4 cells/100 µm^2; HM60+HC vs. HM60 vs. naïve) or tissue water content (83.8 ± 2.6 vs. 85.9 ± 3.6 vs. 81.1 ± 1.9%; HM60+HC vs. HM60 vs. naïve).

Discussion: Our findings indicate that TRPV4 channel blockade attenuates SD propagation and reactive astrogliosis, while it does not influence tissue swelling. These results suggest that TRPV4 is primarily involved in mechanisms related to SD and astrocyte activation, whereas edema formation may depend on distinct pathways. Therefore, TRPV4 inhibition may represent a promising strategy to suppress SD in AIS, but it is unlikely to be sufficient as a standalone anti-edema therapy.

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YP-09 The role of Septin expression and function in human skeletal muscle cell cultures

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Keywords: human skeletal muscle cells, myogenic differentiation, septins, cytoskeleton

Septins are cytoskeletal GTP-binding proteins involved in cell division, membrane organization, cytoskeletal remodeling, and regulating differentiation. It has been previously shown that Septin7 is one of the key proteins in regulating cytoskeletal septin assembly in mouse-originated skeletal muscle cell line, and has a significant impact on physiological organization, force generation, and also on the regeneration program of skeletal muscle in mice. However, their role in human skeletal muscle cells remains insufficiently characterized.

In the present study, we investigated septin expression in human skeletal muscle cell cultures (HSkMc) using both fetal and adult cell lines under proliferative and differentiating conditions. Samples from individual cultures were collected at the proliferative blast stage and at specific phases of differentiation. The gene expression was analyzed by qPCR, while protein expression was examined by Western blot.

Quantitative PCR analysis demonstrated the expression of several septin family members, including Septin2, Septin6, Septin7, and Septin9 in human skeletal muscle cells. Their expression patterns changed between proliferative and differentiated states and also differed fetal and adult cultures. In parallel, the expression of key myogenic markers (*PAX7*, *MYOD*, *MYOGENIN*) varied across differentiation stages, supporting the progression of myogenic differentiation in the examined cultures. Western blot analysis further confirmed the presence of SEPTIN7 and SEPTIN9 proteins in both fetal and adult HSkMc samples. Confocal microscopic analysis of immunostained samples confirmed progressive differentiation in both fetal and adult HSkMc cultures, with clear morphological changes from blast cells to elongated, aligned myotubes during later differentiation stages.

Overall, our results show that specific septins are expressed in human skeletal muscle cell cultures and show stage- and cell type-dependent expression during myogenic differentiation. These findings suggest that septins may contribute to the regulation of human skeletal muscle cell structure and differentiation.

YP-10 Engineering a fluorescent biosensor for detecting endogenous ligands of the orphan receptor GPR84

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Keywords: GPR84; GPCR; fluorescent biosensor; inflammation; ligand discovery

GPR84 is an inflammation-induced G protein-coupled receptor expressed predominantly in myeloid cells and upregulated in inflammatory settings. Although it has been proposed to respond to medium-chain fatty acids, its endogenous ligands and physiological modes of activation remain incompletely understood. Here, we aimed to develop a genetically encoded fluorescent biosensor based on GPR84 to enable visualization of receptor activation in vivo. In parallel, we generated orthologue-specific sensor variants based on the human, mouse, and zebrafish receptors to support the identification of endogenous GPR84 ligands and to enable comparative analysis of receptor activation across species.

Building on our previous GPCR-based biosensor engineering strategy, GPR84-based sensors were generated by inserting cpEGFP - a conformation-sensitive green fluorescent protein variant - into intracellular receptor regions. Sensor architecture was optimized by screening more than 500,000 variants differing in insertion site, linker composition, and cpEGFP design. A first-generation human GPR84 biosensor displayed a ligand-induced fluorescence response with an EC50 of 1.17 ± 0.30 μM.

Receptor activation by the synthetic agonist 6-OAU was validated for human, mouse, and zebrafish GPR84 orthologues using Gqi5-assisted calcium measurements with Fluo-4 in HEK293A cells. These assays confirmed activation of all three orthologues by 6-OAU. Transfer of the optimized linker design to mouse and zebrafish GPR84 yielded functional orthologue biosensors that also responded to 6-OAU.

Together, these results establish GPR84-based fluorescent biosensors as promising tools for visualizing receptor activation and for identifying physiologically relevant GPR84 ligands in inflammatory contexts.

YP-11 Piezo1 activation reduces hyaluronan accumulation in orbital fibroblasts under adipogenic induction

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In thyroid eye disease (TED) enhanced proliferation, increased adipogenesis and hyaluronan (HA) accumulation cause tissue expansion leading to increasing intraorbital pressure that may activate Piezo1 receptors on orbital fibroblasts (OFs). We have previously demonstrated that Piezo1 activation suppresses adipogenesis in TED-derived cells, while this study aimed to investigate its effect on HA production under adipogenic conditions.

Connective tissue derived OFs were isolated from control (non-TED, n=6) and TED patients (n=5). Mechanical stimulation was mimicked by Piezo1 agonist, Yoda1 during a 12-day adipogenic induction. HA levels were quantified by ELISA. Gene expressions of HA metabolism were assessed by RT-PCR, while HA molecular weight distribution was analysed using agarose gel electrophoresis.

Adipogenic stimulation significantly increased HA accumulation both in the culture medium ($p<0.001$) and pericellular coat ($p=0.026$). This effect was attenuated by Yoda1 at both sites ($p=0.005$ and $p=0.001$, respectively) in OFs. Adipogenic conditions upregulated hyaluronan synthase 2 (HAS2) expression ($p=0.011$) and downregulated the expression of the hyaluronidase, cell migration-inducing protein (CEMIP) ($p=0.005$). Piezo1 activation decreased HAS2 ($p<0.0001$) while increasing hyaluronidase-1 (HYAL1) ($p=0.004$) and CEMIP ($p=0.015$) expression. Gel electrophoresis showed that adipogenic stimulation promoted the production of high molecular weight HA ($p<0.001$), whereas Yoda1 treatment reduced high ($p<0.001$) and increased low molecular weight HA ($p=0.003$).

Activation of Piezo1 in OFs not only inhibits adipogenesis but also suppresses HA production under adipogenic conditions. Our findings in non-TED OFs suggest that this HA-modulating effect is independent of adipocyte differentiation. The potential contribution of reduced HA levels to Piezo1-mediated inhibition of adipogenesis warrants further investigation.

YP-12 Proton radiation impairs mechanotransduction in C2C12 Myoblasts

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Keywords: spaceflight, proton radiation, skeletal muscle, microgravity

Skeletal muscle dysfunction during spaceflight arises from combined mechanical and non-gravitational stressors. Here, we modeled solar particle event–like proton irradiation in C2C12 myogenic cells to examine radiation-induced membrane and mechanosensitive channel alterations. Proton exposure increased membrane rigidity and reduced membrane fluidity in a dose-dependent manner and significantly hindered the pharmacological activatability of Piezo1 channels, identifying membrane-mediated mechanotransduction as a potential contributor to radiation-induced muscle dysfunction. Western blot analysis showed no change in the expression levels of the protein. We were able to detect reduced mRNA levels of the channel using qPCR. We also aimed to assess the alterations of certain cytoskeletal proteins caused by proton irradiation. Different subtypes of the Septin family were evaluated. Proton irradiation seemingly changed the dynamic remodeling of Septin5, Septin7 and Septin9. We also simulated a microgravity environment using an AirBus RPM 2.0 clinostat. Our goal was to identify whether *0g* and *0.9g* rotation altered the expression of the key players of myoblast differentiation.

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YP-13 The expression of cytoskeletal septins and SOCE complex proteins during spontaneous muscle regeneration in mdx mice

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Duchenne muscular dystrophy is an X chromosome-linked recessive skeletal muscle disorder caused by the mutation of the *DMD* gene, which encodes the protein dystrophin. Dystrophin is a structural component of the dystrophin-glycoprotein complex, which crosslinks the cytoskeleton with the extracellular matrix. In the absence of functional dystrophin, the sarcolemma becomes highly susceptible to mechanical stress, resulting in micro-injuries upon contraction. The mdx mouse model is the most widely used animal model for studying DMD, carrying a nonsense point mutation which results in the absence of full-length dystrophin. However, mdx mice has milder phenotype caused by a spontaneous regeneration phase between 8-12 weeks of age. During this period, satellite cells are activated and undergo extensive proliferation and differentiation, producing new myofibers; thus, muscle mass is largely restored, and functional capacity is substantially preserved.

Septins are a highly conserved family of GTP-binding proteins, known as the fourth component of the cytoskeleton. Previous research suggests that septins, especially Septin7, are involved in skeletal muscle regeneration and development. Based on available transcriptomic and proteomic analysis data from mdx skeletal muscle samples at different ages, the expression of specific septins, including Septin4, Septin7, and Septin9 is modified compared to the control Bl10 samples. We want to examine whether cytoskeletal septins are involved in the spontaneous regeneration process in young mdx mice.

Our experimental results show that the expression of several septin proteins, as well as the components of store-operated calcium entry, STIM1 and Orai1, are significantly modified during the regeneration phase in 5-, 8-, and 12-week-old mice. These findings indicate that cytoskeletal protein dynamical assembly and changes of SOCE, as part of the calcium homeostasis, are essential mediators of the spontaneous regeneration of skeletal muscle in the dystrophic animal model. Examining the localization of these proteins in *flexor digitorum brevis* single muscle fibers using fluorescent immunostaining, we detected an altered myofibrillar pattern of Septin7, especially near satellite cells.

These results suggest a complex functional role of specific septins in the spontaneous regeneration process of dystrophic skeletal muscle, presumably through the activation of satellite cells, proliferation of committed muscle progenitor cells, and differentiation of myoblasts into multinucleated myotubes.

YP-14 Development and characterization of green and red CXCL12/SDF-1 conformational-sensitive biosensors

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Keywords: GPCR, biosensor, CXCR4, inflammation

The chemokine receptor CXCR4 regulates key physiological processes, including hematopoietic cell retention in the bone marrow, leukocyte migration, and inflammatory responses. It is also essential for HIV entry and is frequently overexpressed in tumor cells, contributing to metastasis formation. Furthermore, mutations in CXCR4 are associated with WHIM syndrome, a rare immunodeficiency disease. Given its involvement in this wide range of pathologies, CXCR4 has become a major focus of drug discovery, with growing interest in its biology. CXCR4 is activated by stromal cell-derived factor-1 (SDF-1/CXCL12), a ligand conserved across species.

In zebrafish, two receptor isoforms (Cxcr4a and Cxcr4b) and two ligand isoforms (Cxcl12a and Cxcl12b) have been identified. Many developmental and immunological processes are predominantly regulated by the Cxcr4b–Cxcl12a signaling axis, including lateral line primordium migration, primordial germ cell guidance, and neutrophil retention at inflammatory sites. Previous studies have demonstrated cross-reactivity between human and zebrafish CXCR4/CXCL12 systems.

Here, we successfully engineered CXCR4-based conformational biosensors by inserting cpEGFP or cpmScarlet into the third intracellular loop (ICL3), a region undergoing activation-dependent conformational rearrangement with transmembrane helix 6. Multiple variants were generated using different insertion sites and linker compositions. A stable library of biosensor constructs was established in HEK293 cells, and ligand-responsive variants were characterized by dose–response analysis to assess sensitivity and dynamic range.

Our aim is to develop green and red biosensors for real-time monitoring of CXCR4 signaling and ligand distribution *in vivo* in zebrafish. Such sensors may also provide a platform for pharmacological profiling and screening of CXCR4-targeting compounds.

Poster Session II. - Immunology

P-23 Increased muscle mass does not prevent subchondral bone sclerosis in a mouse model of established osteoarthritis

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Keywords: osteoarthritis, muscle mass, subchondral bone

Osteoarthritis (OA) is the leading cause of disability, characterized by severe structural changes of the knee joint, including articular cartilage degeneration and subchondral bone remodeling, leading to pain and limited mobility. Disuse muscle atrophy may further aggravate the disease; thus, exercises increasing periarticular muscle mass are frequently recommended as a conservative treatment. However, robust evidence for their efficacy remains limited.

Here, we induced OA by destabilization of the medial meniscus (DMM) in normal C57BL/6 (n = 10) and hypermuscular myostatin-deficient (n = 9) mice, and *in vivo* muscle strength was assessed using grip, hang, and rotarod tests after 8 weeks. Medial tibial plateau subchondral bone microstructure was evaluated via micro-computed tomography. Sham-operated animals were used as controls (normal n = 10; hypermuscular n = 9).

Although body weight and mass of *extensor digitorum longus*, *tibialis anterior*, and *soleus* muscles were higher in hypermuscular mice ($P \leq 0.0157$), DMM and sham-operated animals did not differ significantly within either strain. Hang and grip test performance were higher, while rotarod performance was lower in hypermuscular mice ($P \leq 0.0434$). In normal DMM animals, body-weight-normalized grip force was significantly lower than in sham ($P = 0.0198$). Subchondral bone microstructure showed few differences between normal and hypermuscular animals, whereas both DMM groups exhibited characteristic sclerotic OA changes versus sham, including increased bone volume fraction and trabecular thickness, and decreased bone surface/volume ratio and trabecular pattern factor (all $P \leq 0.0244$). In normal DMM mice, the structure model index was lower than in sham ($P = 0.0023$), while in hypermuscular DMM mice, the degree of anisotropy was higher and the fractal dimension lower than in sham (both $P \leq 0.0101$). These findings indicate increased bone volume and a denser, more connected trabecular structure in OA, independently of muscle mass.

These results suggest that hypermuscularity does not mitigate subchondral bone alterations in established OA in the DMM model at 8 weeks. Muscle mass and *in vivo* performance were largely preserved, highlighting a potential early therapeutic window before muscle atrophy or weakening due to disuse becomes established.

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P-24 ARHGAP25 regulates hematopoietic cell-dependent inflammation in the imiquimod-induced psoriasiform dermatitis model

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Keywords: ARHGAP25, psoriasis, imiquimod-induced mouse model, immune cell infiltration

Background: ARHGAP25 is a leukocyte-specific GTPase-activating protein that plays an important role in the regulation of immune cell function and inflammatory responses. Psoriasis is a chronic immune-mediated skin disease with a complex and not yet fully understood pathogenesis. Given the regulatory role of ARHGAP25 in inflammation, it may also contribute to the development and severity of psoriatic skin inflammation.

Aims: This study aimed to investigate the role of ARHGAP25 in the pathogenesis of psoriasis using the imiquimod (IMQ)-induced psoriasiform mouse model, with particular focus on disease severity and the contribution of hematopoietic cells.

Methods: The experiments were performed using wild-type (WT), ARHGAP25-deficient (KO), and bone marrow chimeric (WT→WT, KO→WT,WT→KO, KO→KO) mice were used in our experiments. Psoriasiform inflammation was induced by daily topical IMQ treatment for 6 consecutive days, while control animals received Vaseline. Disease progression was monitored by measuring skin thickness and scoring erythema, scaling, and induration. Following the treatment period, animals were further monitored for an additional 10–11 days to assess the resolution phase. To evaluate immune cell infiltration, skin samples were collected on days 4, 6, and 10. Infiltration was analyzed by **flow cytometry** and **hematoxylin and eosin staining**, and **quantitative PCR (qPCR) analyses** are planned to determine the expression of inflammatory markers.

Results: IMQ treatment induced pronounced inflammation in all experimental groups. ARHGAP25-deficient mice exhibited more severe clinical symptoms compared to WT animals, reflected by increased erythema and scaling scores. These findings were partially recapitulated in bone marrow chimeric mice, suggesting that hematopoietic cells contribute to the observed phenotype. Ongoing analyses of skin-infiltrating immune cells are expected to provide further insight into the cellular mechanisms underlying these differences.

Conclusions: Our results suggest that ARHGAP25 plays an anti-inflammatory role in the IMQ-induced psoriasiform dermatitis model, at least in part through hematopoietic cell-dependent mechanisms.

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P-25 Beyond classical pH regulation: bicarbonate enhances key effector functions of human neutrophils

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Keywords: Bicarbonate, Neutrophils, Phagocytosis

Background: Recent studies have suggested that bicarbonate has far more diverse effects than a simple buffer system. It has been described that it inhibits the growth and biofilm formation of various pathogenic bacteria, increases the activity of antimicrobial peptides, and enhances bacterial susceptibility to certain antibiotics. Although HCO_3^- has been shown to regulate neutrophil extracellular trap (NET) formation, there are no published data about its effects on other important neutrophil functions. Therefore, in our current study, we aim to investigate the impact of HCO_3^- on these functions using human polymorphonuclear leukocytes (PMNs).

Methods: PMNs were isolated from the blood of healthy adult volunteers. The phagocytosis of GFP-expressing, serum opsonized *S. aureus*, and F-actin remodeling during phagocytosis were detected with flow cytometry. Superoxide production of neutrophils was measured with lucigenin luminescence assay upon opsonized zymosan stimulus. The *S. aureus* killing capacity of PMN was assessed using the bacterial survival assay. Specific inhibitors were used to reveal the uptake mechanisms and the related phagocytosis-modulating effect of bicarbonate. In each set of experiments, the following experimental conditions were used: plain Hank's Balanced Salt Solution (HBSS), or HBSS supplemented with 25 or 50 mM $NaHCO_3$ in a 5% CO_2 environment, and as adequate controls, plain HBSS or HBSS supplemented with 25 or 50 mM NaCl in an ambient air environment.

Results: Both 25 and 50 mM NaHCO3 significantly increased the phagocytic capacity of neutrophils and enhanced F-actin polymerization during phagocytosis, in a pH-independent manner, compared to osmotic controls. Intracellular superoxide production and bacterium-killing capacity of PMN were also elevated by HCO_3^-. Specific inhibition of NBC transporters, but not of the carbonic anhydrase enzyme decreased the modulating effect of bicarbonate.

Conclusions: Apart from the previously described antibacterial properties of bicarbonate, our data suggest that HCO_3^- stimulates important effector functions of PMNs. Following uptake via NBC transporters, HCO_3^- enhances the phagocytosing efficiency of neutrophils by increasing actin polymerization. These findings suggest that bicarbonate may represent a useful adjunctive approach in infectious conditions where efficient neutrophil-mediated bacterial clearance is critical, such as cystic fibrosis.

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P-26 Green-synthesized silver nanoparticles as an alternative antibacterial strategy against antibiotic-resistant bacteria in dairy cattle

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Background: The increasing prevalence of antibiotic-resistant bacteria represents a major challenge in both human and veterinary medicine, emphasizing the urgent need for alternative antimicrobial strategies. Green synthesis of nanoparticles using plant extracts offers an environmentally friendly and cost-effective approach, in which bioactive compounds act as reducing and stabilizing agents.

Aims: This study aimed to synthesize rosemary-mediated silver nanoparticles (AgNPs-RO) using *Rosmarinus officinalis* extract and to evaluate their antibacterial activity against antibiotic-resistant bacteria isolated from dairy cattle.

Methods: Silver nanoparticles were synthesized via green synthesis using rosemary extract. Their formation was confirmed by UV–Vis spectroscopy through the detection of a characteristic surface plasmon resonance band. Morphology and dispersion were analyzed using scanning electron microscopy (SEM), while particle size distribution was determined by image analysis. Elemental composition was verified by energy-dispersive X-ray spectroscopy (EDX). Antibacterial activity was assessed using the disk diffusion method against *Escherichia coli*, *Streptococcus uberis*, and *Staphylococcus aureus* isolated from dairy cow milk. Gentamicin was used as a positive control and distilled water as a negative control.

Results: The synthesized AgNPs-RO exhibited antibacterial activity against all tested strains. Inhibition zones reached 10 mm for *E. coli* and 6 mm for both *S. uberis* and *S. aureus*. In contrast, gentamicin showed no inhibitory effect against the resistant strains.

Conclusion: These findings suggest that green-synthesized AgNPs-RO represent a promising alternative or complementary antimicrobial strategy in veterinary medicine. Further research will focus on optimizing synthesis parameters, improving nanoparticle stability, and investigating their mechanisms of action and applicability under field conditions.

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P-27 The effect of synovial fibroblast-specific Syk deletion in a chronic polyarthritis model in mice

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Keywords: rheumatoid arthritis, synovial fibroblast, tyrosine kinase

Introduction: Rheumatoid arthritis causes synovitis and joint destruction, which is partly mediated by synovial fibroblasts (FLS). Since many patients do not respond to current available therapies, novel therapeutic targets and drugs must be identified. Syk is an intracellular tyrosine-kinase that is present in synovial fibroblasts, has been shown to contribute to the development of experimental autoimmune arthritis, while its expression is upregulated in the rheumatoid arthritis synovium.

Aims: Here, we investigated the role of the Syk tyrosine-kinase in synovial fibroblasts in the development of a chronic experimental arthritis.

Methods: FLS-specific deletion of Syk was achieved by the Cre-Lox system (resulting in $Syk^{\Delta FLS}$ mice). We induced chronic polyarthritis by ovalbumin-specific T cell injection and repeated ovalbumin exposure in wild type and $Syk^{\Delta FLS}$ mice. Arthritis was followed by ankle thickness measurement, clinical scoring and a functional test. Pannus formation was examined by histological analysis, while local immune cell accumulation was measured by flow cytometry. Proinflammatory cytokine levels and autoantibody titers were detected by ELISA. The presence of bone erosions was examined by micro-CT analysis.

Results: $Syk^{\Delta FLS}$ mice developed milder joint inflammation compared to wild type animals. In line with these findings, pannus formation was less severe, immune cell accumulation and proinflammatory cytokine levels were also lower in $Syk^{\Delta FLS}$ mice. Moreover, FLS-specific Syk expression was critical for the development of bone erosions and autoantibody production.

Conclusions: Our results indicate that Syk expression in synovial fibroblasts is important for the development of experimental chronic arthritis. These data strengthen the hypotesis that Syk inhibition may have a beneficial effect in the treatment of human autoimmune arthritis.

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P-28 The role of a C-type lectin receptor in immune complex-mediated cell responses of phagocytes

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Keywords: C-type lectin receptor, immune complex, phagocyte

Among other C-type lectin receptors, the expression of Dectin-2 showed upregulation in (experimental) immune complex-mediated epidermolysis bullosa acquisita (EBA). In line with this finding, we have found that Dectin-2 knockout (Dectin-2$^{-/-}$) mice were almost completely protected against experimental EBA, where the accumulation of neutrophils and macrophages at the site of inflammation were dramatically decreased in Dectin-2$^{-/-}$ mice compared to wild type animals. Here, we investigated which phagocytes require Dectin-2 expression for their in vitro cell responses.

We used wild type and Dectin-2$^{-/-}$ mice. Peripheral and bone marrow neutrophil and macrophage counts and cell surface expression of crucial receptors were measured by flow cytometry. Freshly isolated mouse neutrophils or cultured bone marrow-derived macrophages were activated by immune complex surfaces in vitro. Superoxide release of neutrophils was measured by a cytochrome c reduction test, while production of reactive oxygen species by macrophages was detected by a chemiluminescence method. In vitro cytokine levels were measured by ELISA. Neutrophil migration was tested in the in vitro Transwell assay.

The absence of Dectin-2 did not affect the bone marrow or the circulating phagocyte cell numbers. The maturation and the expression of cell surface markers (e.g. Fc receptors, integrins) were unaffected by Dectin-2-deficiency. Immune complex-activated Dectin-2$^{-/-}$ neutrophils showed significantly decreased superoxide and cytokine release in contrast to wild type cells, while their in vitro migration was not impaired. On the other hand, immune-complex-mediated cell responses of Dectin-2$^{-/-}$ macrophages were comparable to wild type cells.

Our results indicate that the essential role of Dectin-2 in the development of autoimmune dermatitis is probably due to the importance of this receptor in immune complex-mediated neutrophil functions. Our findings help us to better understand the pathomechanism of Fc receptor-mediated autoimmune skin diseases, while Dectin-2 may serve as a potential therapeutic target in these disorders in the future.

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P-29 Intracerebroventricular and intranasal application of alarin reduces body weight in male rats

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Keywords: obesity, anti-obesity target, alarin, body weight regulation

Obesity represents a major public health burden, necessitating novel therapeutic targets. Alarin, one of the newest members of the galanin neuropeptide family, may be a potential candidate for anti-obesity therapy. Alarin has been shown to reduce food intake and increase energy expenditure when administered centrally, but its long-term effects on body weight remain unclear. Here, we examined the impact of a 7-day intracerebroventricular alarin infusion on energy homeostasis. Because intranasal delivery is a clinically feasible route for delivery of centrally acting anorexigenic peptides, we also assessed the acute effects of intranasal alarin on food intake and body weight.

Male Wistar rats received chronic central alarin infusion, while food intake, body weight, heart rate (indicating metabolic rate), locomotor activity, and body temperature were continuously monitored via biotelemetry. FOSB immunohistochemistry was performed in the central amygdala and median and medial preoptic nuclei. Acute effects of intranasal alarin and the potential antagonistic action of its truncated analogue, 6–25Cys-alarin were tested in ad libitum–fed and 24-h–fasted rats using an automated FeedScale system.

Central alarin infusion increased FOSB-immunoreactive cell numbers in the amygdala and the medial preoptic nuclei, indicating neuronal activation. Alarin significantly reduced food intake and increased heat production resulting in weight loss. Intranasal alarin also decreased body weight. Its anorexigenic effect was antagonized by 6-25Cys-alarin.

Alarin acts as a catabolic mediator in the long-term regulation of energy balance and body weight. Intranasal delivery of alarin represents a promising, clinically relevant therapeutic option for obesity treatment.

P-30 Lymphatic ablation drives severe autoimmune arthritis via immune-complex–mediated inflammation

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Keywords: lymphatics, serum transfer arthritis, neutrophil granulocytes, bone erosion, immune complexes, inflammation

Rheumatoid arthritis is a chronic autoimmune disorder, primarily affecting the small joints. Despite the known connection between the immune and lymphatic systems, the role of lymphatics in autoimmune arthritis remains unclear.

Here, we aimed to investigate the mechanisms by which lymphatic vessels influence the effector phase of autoimmune arthritis.

Diphtheria toxin-mediated ablation of the local lymphatic vasculature was induced in the hindlimbs of a transgenic mouse model, followed by the induction of arthritis via K/BxN serum transfer. Disease progression was monitored by ankle thickness measurement and clinical scoring. Hindlimb samples were processed for histology, and peripheral autoantibody and local immune-complex levels were measured by ELISA. Local immune cell populations were quantified by flow cytometry, and arthritis-associated bone alterations were assessed by micro-CT.

Lymphatic-deficient mice developed arthritis earlier and displayed more severe progression compared with controls. Histological and micro-CT analysis revealed significantly exacerbated bone erosion and structural damage in lymphatic-deficient arthritic mice. Peripheral autoantibody levels showed no significant differences between arthritic groups. In contrast, local immune-complex accumulation was markedly elevated in lymphatic-deficient arthritic mice, accompanied by a substantial increase in neutrophil infiltration.

Our findings indicate that the local lymphatic vasculature plays a critical immunomodulatory role in the effector phase of autoimmune arthritis. Loss of lymphatic vessels exacerbated autoimmune arthritis, possibly driven by impaired clearance. These results suggest that therapeutic strategies aimed at preserving or enhancing lymphatic function may hold promise in the treatment of autoimmune arthritis.

P-31 Investigation of CRISPR editing in hematopoietic cells

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Introduction: Due to the recognition of its therapeutic potential, the CRISPR-Cas9 system has become a primary tool for human gene therapy efforts. However, detailed characterization of efficiency and potential complications is a prerequisite for translational application.

Objectives: Our study aimed to provide a precise characterization of the CRISPR-Cas9 system's operation in a therapeutically relevant model system by investigating targeted gene knockouts in a mouse hematopoietic progenitor cell line.

Methods: Gene editing was performed in progenitor cells constitutively expressing Cas9, using lentiviral transduction of gRNAs targeting three specific genes: *Itgb2*, *Fcer1g*, and *Cybb*. The efficiency of the knockout was verified by measuring target protein levels using flow cytometry. Genetic alterations were first analysed by TOPO cloning and sequencing of PCR products from the targeted locus. For high-throughput analysis, NG sequencing of PCR amplicons from the edited gene regions was used. For further mutational analysis and direct genotype-phenotype correlation, single-cell clones were established from the edited heterogeneous population and analyzed by Sanger sequencing.

Results: Flow cytometry of the polyclonal population showed that the target protein was almost entirely absent from the edited cells. In the 103 established clones, gene knockout efficiency also proved to be nearly 100%; normal protein expression was detected in only a single case, where the integration of a mutant gRNA was confirmed. The resulting mutations were typically deletions, which in some cases extended to several hundred base pairs; consequently, these would not have been detectable by NGS sequencing of short amplicons alone. We also examined the factors influencing the speed of gene knockout. Testing five different gRNA sequences, deletion of the *Itgb2* gene could be completed in 5 days using the most efficient guide, while other gRNAs required significantly more time. In contrast, when examining the homozygous and heterozygous status of the Cas9 transgene, the knockout speed proved to be independent of gene dosage.

Conclusion: Efficient gene editing can be achieved in hematopoietic cells using the CRISPR-Cas9 system. The dominant genetic consequence is deletion, which in many cases results in the loss of segments spanning several hundred base pairs. The guide RNA sequence significantly determines the time kinetics of the knockout, whereas the copy number of the Cas9 gene does not alter editing efficiency. Based on our results, while the efficiency of the CRISPR-Cas9 method is confirmed in the investigated cell type, the significant genetic changes occurring at the targeted loci raise the possibility of potential genotoxic complications.

P-32 Endogenous hydrogen sulfide protects pancreatic acinar cells from acute pancreatitis-inducing agents

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Introduction: Human cells produce hydrogen sulfide (H_2S) through the enzymes cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CTH), and 3-mercaptopyruvate sulfurtransferase (MPST), where it plays a substantial role in cell signalling. Although the physiological and pathophysiological effects of H_2S in the pancreas have been investigated, many questions and apparent contradictions remain regarding how it acts in acute pancreatitis (AP).

Aims: To determine the expression and role of H_2S-producing enzymes in pancreatic acinar cell injury caused by AP-inducing agents.

Methods: Mouse primary pancreatic acinar cells were isolated with collagenase digestion. The gene expression levels of *Cbs*, *Cth*, and *Mpst* were determined by qPCR. Cells were treated with AP-inducing agents (cerulein, ethanol and palmitoleic acid, chenodeoxycholate) and CTH or MPST inhibitors. *In vitro* assays were utilised to determine cellular viability (calcein-AM, propidium iodide − PI) and levels of intracellular reactive oxygen species (ROS).

Results: Pancreatic acinar cells showed the highest mRNA expression for *Cth*, followed by *Cbs* and *Mpst*. AP-inducing agents reduced the cellular metabolic activity (calcein-AM) and caused necrosis (PI). Cystathionine-γ-lyase-IN-1 (CTH inhibitor) and I3MT-3 (MPST inhibitor) exacerbated the effects of AP-inducers in almost all cases by increasing cellular toxicity and decreasing acinar viability. Administration of the inhibitors did not influence the elevated ROS production promoted by the aforementioned AP-inducers. CTH or MPST inhibitors alone caused no adverse effects on cells.

Conclusion: Our findings suggest that H_2S contributes to cytoprotection without affecting the elevated ROS production in AP, highlighting the need for further investigation into its role in AP.

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P-33 Investigating the role of β2-integrins in *in vitro* and *in vivo* inflammatory reactions

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Keywords: neutrophil granulocytes, autoimmun disease, inflammatory

Neutrophil granulocytes (neutrophils) possess well-known adhesion molecules called integrins, which actively induce various signaling processes. Integrins facilitate cytoskeletal rearrangement, regulate gene expression and establish cell differentiation in an adhesion-dependent manner, thereby activating several signal pathways. The role of β2 (CD18-containing) integrins expressed on the cell surface of neutrophils, such as LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) have been proposed to play an important role in the development of the autoimmune inflammatory cascade. However, their specific roles are incompletely understood.

Our aim was to test the role of LFA-1 and Mac-1 in various in vitro and in vivo aspects of the overall inflammation process.

Bone marrow cells from wild-type (WT), CD11a-deficient (LFA-1 KO), CD11b-deficient (Mac-1 KO) or CD18-deficient (CD18 KO) mice were transplanted into lethally irradiated wild-type recipients to generate the respective bone marrow chimeras. Expression of the same molecules on the surface of neutrophils was tested by flow cytometry. K/BxN serum-transfer arthritis was investigated in all chimeras. Disease development was followed by testing macroscopic signs of arthritis. Further analyses of cell surface marker expression were performed by flow cytometry from peripheral blood samples. We have also tested the superoxide release and Transwell migration of neutrophils from the same mice.

While WT chimeras developed robust K/BxN serum-transfer arthritis, practically no arthritis developed in CD18 KO chimeras. LFA-1 KO chimeras were partially protected from arthritis development, especially at lower doses of K/BxN serum. Mac-1 KO chimeras even showed a slightly more severe arthritis disease course. We have observed substantial reduction of adhesion-dependent superoxide release in CD18 KO and Mac-1 KO neutrophils. On the other hand, LFA-1 KO neutrophils showed impaired in vitro migration.

Though our results suggest a critical role for CD18 in various in vivo and in vitro inflammatory reactions, the contribution of LFA-1 and Mac-1 appears to differ in the different functional readouts. While LFA-1 but not Mac-1 appears to mediate K/BxN serum-transfer arthritis and in vitro migration, Mac-1 is more involved in the respiratory burst response of adherent neutrophils.

P-34 Anti-ferroptotic compounds protect pancreatic acinar cells against agents inducing acute pancreatitis

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Introduction: Acute pancreatitis (AP) is an inflammatory condition of the pancreas. There is no specific treatment, and the mortality rate can reach up to 50%. Recent studies have identified ferroptosis, a novel form of regulated cell death associated with the progression of AP. This non-apoptotic cell death is characterized by severe lipid peroxidation and is primarily triggered by excessive iron accumulation and reactive oxygen species (ROS).

Aims: We aim to test antioxidant compounds on primary pancreatic acinar cells to evaluate their ability to reduce ferroptosis caused by AP-inducing agents.

Methods: Primary pancreatic acinar cells were isolated with collagenase digestion and used for *in vitro* measurements. Cells were treated for 8 hours with AP- or ferroptosis-inducing solutions to evoke cellular damage. Co-treatments with antioxidants were performed. Cellular viability was assessed using propidium iodide (PI) and calcein-AM assays, while intracellular ROS levels were measured with a carboxy-H2DCFDA-based method.

Results: Treatments with the antioxidants (ferrostatin-1, liproxstatin-1, or Trolox) showed no adverse effects on acinar cells. AP- (chenodeoxycholate; ethanol+palmitoleic acid) and ferroptosis-inducing (1S,3R-RSL 3; erastin) molecules decreased cellular viability (calcein-AM) and induced toxicity (PI). We demonstrated that all antioxidant treatments markedly improved the impairement in cellular metabolic activity and toxicity caused by AP- and ferroptosis-inducing agents. Both liproxstatin-1 and ferrostatin-1 significantly reduced ROS levels when acinar cells were treated with AP- and ferroptosis-inducing compounds.

Conclusion: Antioxidant compounds have been shown to reduce oxidative stress and protect pancreatic acinar cells from toxic treatments, indicating cytoprotective effects that may help mitigate ferroptosis in acute pancreatitis.

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P-35 ARHGAP25 regulates thymic egress and peripheral T-cell homeostasis

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Keywords: thymocyte, thymic egress, ARHGAP25

ARHGAP25 is a Rac-specific GTPase-activating protein involved in several immune processes. While its role in B-cell biology has recently gained attention, its contribution to T-cell biology remains poorly understood. In this study, we investigated the role of ARHGAP25 in thymocyte maturation and thymic egress.

Adult Arhgap25+/+ wild-type (WT) and Arhgap25−/− knockout (KO) mice on a C57BL/6 background were analyzed for T-cell numbers in peripheral blood, spleen, and lymph nodes, as well as for thymus size and cellular composition. Isolated thymocytes were further examined to explore the molecular mechanisms underlying the observed alterations.

KO mice showed reduced T-cell counts in peripheral blood. Similar changes were observed in peripheral lymph nodes, which were also smaller in size. In contrast, the spleen was not markedly affected. The thymus of KO animals was enlarged, with increased thymocyte numbers and altered subpopulation ratios. Notably, KO thymi contained a higher proportion of mature thymocytes, with increased CD69 and reduced S1PR surface expression, indicating impaired thymic egress. As ARHGAP25 is a Rac-specific GTPase-activating protein, the elevated Rac activation and ERK phosphorylation detected in KO thymocytes suggest that ARHGAP25 regulates thymocyte exit through a Rac-ERK-dependent pathway that influences the S1PR/CD69 axis.

These findings identify ARHGAP25 as an important regulator of thymic egress and peripheral T-cell homeostasis. Our results support a model in which loss of ARHGAP25 disrupts Rac-ERK signaling, alters the S1PR/CD69 balance required for thymocyte emigration, and thereby causes thymic retention of mature thymocytes with reduced peripheral T-cell counts.

P-36 Effect of prostaglandin analogues on hyaluronan accumulation in orbital fibroblasts

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Prostaglandin analogues (PGAs): latanoprost, tafluprost and bimatoprost are the first line treatment in glaucoma. The long-term use of PGAs results in fat reduction via adipogenesis inhibition which, together with hyaluronan accumulation, is characteristic of thyroid eye disease (TED). This study explores the connection between PGA treatment and HA metabolism.

Primary orbital fibroblasts (OFs) originated from TED (n=4) and non-TED (n=4) connective tissues were used. OFs were cultured with or without a PGA or prostaglandin F2α (PGF2α), in the presence or absence of PDGF-BB (10 ng/ml) stimulation. HA was measured by ELISA. The mRNA expressions of hyaluronan synthases (HAS1, HAS2 and HAS3) and hyaluronidases (HYAL1, HYAL2 and CEMIP) were examined by RT-PCR.

Through the 72h treatment, the PGAs reduced the HA content in both the culture media and the pericellular coat of OFs ($p<0.001$). While latanoprost was the only PGA to reduce HAS2 mRNA levels ($p<0.001$), all tested PGAs and PF2α significantly up-regulated CEMIP mRNA expression ($p<0.001$). PGAs elicited diverse responses in HA synthesis following PDGF-BB stimulation. Latanoprost and tafluprost reduced the HA production to or below the untreated level ($p<0.0001$). Each PGAs and PF2α consistently suppressed HA production in the pericellular coat ($p<0.0001$) under PDGF-BB stimulation. Only latanoprost was able to suppress the stimulated HAS2 expression ($p<0.001$).

The findings uncover the specific molecular mechanism underlying PGAs' effect on HA turnover in OFs. Beyond secondary glaucoma, HA accumulation, a key driver of TED progression is modulated by PGAs. This may represent a potential therapeutic strategy for TED.

P-37 Investigation of tissue-specific mitochondrial dysfunctions and their interrelationships in experimental sepsis

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Introduction: Sepsis is a life-threatening condition characterized by dysregulated systemic inflammation and progressive multiorgan dysfunction, in which mitochondrial damage plays a central role. However, the possible organ-specific relationships between mitochondrial function and systemic pathophysiology remain incompletely understood. In this study, we aimed to investigate correlations between mitochondrial respiration and organ dysfunction in a rat model of experimental sepsis.

Methods: Sepsis was induced in male Sprague Dawley rats (380-420g) by intraperitoneal fecal inoculum, and organ function was assessed using mean arterial pressure, PaO_2/FiO_2 ratio, serum urea, lactate, and alanine aminotransferase, while inflammatory status was evaluated via interleukin-6 and endothelin-1 levels ($n=35_{septic}$, $35_{control}$). Mitochondrial function in liver, cerebellum, and hippocampus was measured using high-resolution respirometry, quantifying oxidative phosphorylation (OXPHOS), substrate oxidation, leak respiration, and respiratory control ratios for Complex I and II. A preliminary diagnostic power analysis using receiver operating characteristic curves revealed that substrate oxidation (AUC_{liver} = 0.90; $AUC_{cerebellum}$ = 0.65; $AUC_{hippocampus}$ = 0.61) and OXPHOS (AUC_{liver} = 0.91; $AUC_{cerebellum}$ = 0.82; $AUC_{hippocampus}$ = 0.81) were the most reliable discriminators among mitochondrial parameters across the investigated organs, these parameters were therefore selected for subsequent correlation analyses.

Results: In the septic animals, all parameters of organ dysfunction and inflammation showed significant deterioration. Correlation analysis revealed a strong association between mitochondrial reactions in different brain regions: complex II-linked cerebellar and hippocampal OXPHOS showed a significant positive correlation ($r = 0.629$, $p < 0.001$). In contrast, correlations between liver and brain mitochondrial parameters were weak and non-significant, with liver–cerebellum $r = –0.054$ ($p = 0.757$) and liver–hippocampus $r = –0.208$ ($p = 0.230$), indicating relative independence of hepatic mitochondrial function from central nervous system energetics. Hippocampal OXPHOS negatively correlated with mean arterial pressure ($r = –0.32$), suggesting perfusion-dependent vulnerability.

Conclusion: These findings demonstrate that focusing on parameters with high diagnostic power enables the identification of biologically meaningful correlations. The findings suggest that mitochondrial impairment in specific brain regions occurs independently of the abnormalities detected in the liver. This supports the concept of organ-specific mitochondrial vulnerability as both a consequence and a potential driver of multiorgan failure in sepsis. Importantly, these observations also highlight mitochondrial respiration as a potential therapeutic target in sepsis, suggesting that interventions aimed at preserving or restoring organ-specific mitochondrial function may represent a promising strategy to mitigate multiorgan dysfunction.

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P-38 Respiratory burst and degranulation of neutrophil-like cells differentiated from CRISPR/Cas9 modified conditionally immortalized myeloid progenitors

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Keywords: respiratory burst, degranulation, HoxB8 cells

Neutrophils are difficult to be manipulated by genetic approaches. This has been overcome by conditional immortalization of mouse myeloid progenitors (HoxB8 progenitors), from which HoxB8 neutrophil like cells (NLCs) can be differentiated. We have set up a system for the CRISPR/Cas9-based genetic manipulation of HoxB8 progenitors and were able to delete several isoforms of a protein family, including the PKC-α (Prkca CRKO), the PKC-β (Prkcb CRKO), the PKC-δ (Prkcd CRKO), and the PKC-ε (Prkce CRKO).

The aim of our experiments was to investigate the respiratory burst and the degranulation of the various CRKO HoxB8 NLCs.

HoxB8 NLCs were generated by estrogen withdrawal along with G-CSF treatment for 4 days. For the respiratory burst experiments, the cells were stimulated by immobilized IgG immune complexes, proinflammatory agonists on an integrin ligand surface, or by PMA stimulus. Superoxide production was measured by a cytochrome c reduction assay. For the other respiratory burst assay and degranulation experiments, the cells were stimulated by fMLP, or by PMA stimulus. Reactive oxygen species (ROS) production and degranulation was measured by flow cytometry.

Wild-type HoxB8 NLCs were able to produce superoxide upon various stimulation. Upon these stimulation, Prkcb CRKO and Prkcd CRKO NLCs showed reduced superoxide production. Wild-type, Prkca CRKO, Prkce CRKO NLCs were able to produce ROS upon PMA stimulation, but in case of Prkcb CRKO and Prkcd CRKO HoxB8 NLCs the ROS production was decreased. The wild-type, Prkca CRKO, and Prkce CRKO NLCs exhibited a normal degranulation response, whereas the degranulation reaction was reduced in Prkcb CRKO and Prkcd CRKO NLCs.

Our results indicate that PKC-β and PKC-δ may play a role in the respiratory burst and degranulation response of HoxB8 NLCs. Therefore, our CRISPR-based targeting strategy is suitable for deleting functionally relevant proteins from NLCs.

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P-39 Investigation of therapeutic complement inhibitors on blister formation in bullous pemphigoid

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Introduction: Bullous pemphigoid (BP) is the most common autoimmune blistering skin disease, characterized by autoantibody-depositions at the dermo-epidermal junction of the skin, inducing myeloid cell infiltration and dermo-epidermal separation. The autoantibody deposition is also known to be followed by the activation of the complement system; however, its functional significance remains unclear, and anti-complement therapies are not currently used in clinical practice for BP.

Aim: We aimed to investigate the effect of therapeutic complement-inhibiting antibodies on blister formation in a fully human model of BP.

Methods: Healthy human skin were used to prepare frozen sections, which were incubated with sera from BP patients. We then incubated the sections with human plasma and performed complement (C3 and C5) immunofluorescence staining. To induce blister formation, we coincubated the BP-serum-treated sections with human granulocytes and plasma, and examined dermo-epidermal separation after haematoxylin-eosin staining. We investigated the effects of two C5-inhibiting therapeutic antibodies, eculizumab and ravulizumab, across a wide range of concentrations, as well as at therapeutic concentrations.

Results: Both eculizumab and ravulizumab effectively inhibited C5 deposition at a concentration of 20 μg/mL, whereas had no effect on C3 deposition. Regarding dermoepidermal separation, both therapeutic antibodies effectively inhibited skin separation with a half-maximal inhibitory concentration (IC50) of 3.6 μg/mL for eculizumab and 5.4 μg/mL for ravulizumab. To further investigate this, we examined the plasma of four patients (three treated with eculizumab and one with ravulizumab) in this model. Plasma from the three eculizumabtreated patients inhibited dermo-epidermal separation even at a 5-fold dilution with untreated plasma, while plasma from the patient treated with ravulizumab resulted in nearly complete inhibition even at a 20-fold dilution.

Conclusion: Taken together, our experiments have successfully demonstrated the efficacy of two clinically used complement inhibitors in a fully human BP model. These findings suggest an important role for the complement system in BP pathogenesis and may contribute to the initiation of clinical trials for this difficult-to-treat disease.

P-40 CRISPR-engineered HoxB8 cells for the analysis of PKC isoforms in neutrophil antibacterial responses

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Keywords: HoxB8, CRISPR/Cas9, neutrophil, antibacterial response

In conditionally immortalized HoxB8 progenitors, the estrogen-dependent HoxB8 transcription factor maintains the cells in a myeloid progenitor stage. Upon estrogen withdrawal, HoxB8 progenitors differentiate into neutrophil-like cells (HoxB8 NLCs), offering a suitable framework for genetic modification and functional analysis of the neutrophil lineage. These progenitors were subjected to targeted CRISPR/Cas9 gene editing to disrupt the genes encoding the nine known PKC isoforms (Prkc CRKOs).

Phagocytosis of normal murine serum-opsonized or non-opsonized GFP-expressing Staphylococcus aureus was followed by flow cytometry. Bacterial survival was tested using the AlamarBlue cell viability assay or by quantifying colony-forming units. Spreading was tested by stimulating cells with TNFα or Pam3CSK4 on a fibrinogen surface, or by plating cells on immobilized IgG immune complexes without additional stimulus. Cell shape changes are assessed by phase contrast microscopy. 2D chemotactic migration of HoxB8 NLCs was tested in fibrinogen-coated IBIDI µ-slide Chemotaxis chambers towards an fMLP gradient by analyzing single-cell trajectories.

Wild type (Empty Vector transduced, EV) HoxB8 NLCs were able to spread over the fibrinogen surfaces, or over an immobilized IgG immune complex surface. EV NLCs were able to effectively migrate towards fMLP gradient and were able to show antibacterial functions against Staphylococcus aureus bacteria of the USA300 strain. Disrupting a single Prkc isoform-encoding gene did not affect neutrophil spreading on fibrinogen surfaces upon TNFα or Pam3CSK4 stimulation. Prkcd CRKO cells showed partially reduced spreading ability on immune complex surfaces. Disruption of a single Prkc gene is insufficient to completely abolish antibacterial functions.

P-41 A novel fully human *ex vivo* model to study granulocyte migration in bullous pemphigoid

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The deposition of autoantibodies produced during bullous pemphigoid (BP) results in complement deposition, myeloid cell infiltration, degradation of the dermal-epidermal junction (DEJ), and the formation of tense blisters. Previous research in human models suggested the important role of Fcγ receptors and complement proteins in blister formation, however their exact pathomechanistic role remained unclear.

We aimed to set up an ex vivo migration model of BP incorporating automated singlecell tracking analysis and to evaluate the effects of FcγRIII and complement C5 inhibition in this new system.

Frozen sections of healthy human skin were treated with heat-inactivated serum from BP patients, then incubated with healthy human plasma and fluorescently labelled granulocytes. Cell migration was assessed with real-time fluorescent microscopy. Single-cell tracking analysis was performed using Fiji and automated using Trackpy. We examined the effects of anti-FcγRIII antibodies and eculizumab (anti-C5 antibody) on granulocyte migration analysing general and BP specific migration parameters.

We successfully set up the model and automated the single-cell tracking analysis, validating its accuracy. Granulocytes exhibited intensive chemokinesis and, on BP serumtreated sections accumulated along the DEJ. Here, single-cell tracking revealed pronounced granulocyte chemotaxis towards the DEJ, most prominent within 150µm of the DEJ. AntiFcγRIII antibodies did not affect granulocyte chemotaxis. Eculizumab treatment strongly decreased granulocyte accumulation and DEJ-directed movement without affecting overall cell motility.

Our human *ex vivo* migration model, utilizing single-cell tracking, enables pharmacological study of myeloid cell infiltration in BP. Furthermore, our eculizumab findings show that complement-mediated chemotaxis drives pathogenesis, explaining how the drug inhibits blister formation.

P-42 The effect of Syk inhibition on inflammatory processes induced by monosodium urate crystals

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Introduction: Gouty arthritis is a common inflammatory disease triggered by the deposition of monosodium urate (MSU) crystals in the joints and surrounding tissues, primarily mediated by phagocytic cells. Here we investigate the effects of entospletinib, a well-known inhibitor of Syk tyrosine kinase, on MSU crystal-induced inflammatory processes in vitro and in vivo.

Method: Wild-type, vehicle-, or entospletinib-treated bone marrow-derived murine or human neutrophils were stimulated with MSU crystals. We monitored reactive oxygen species production by luminometric assay and inflammatory mediator release by ELISA method. In our gouty arthritis model, wild-type animals were treated with 50 mg/kg of entospletinib or its vehicle via oral gavage. Arthritis was induced by injecting MSU crystals into the right hind paw or PBS into the left hind paw of the mice as a control.

Results: MSU crystals evoked robust ROS production and proinflammatory cytokine and chemokine release in vehicle-treated cells, while entospletinib treatment effectively inhibited these cell responses in a dose-dependent manner. Almost complete inhibition of the urate crystal-induced respiratory burst, as well as IL-1β and MIP-2 release from neutrophils, was achieved with 1 μM entospletinib. MSU crystal-induced paw swelling, leukocyte infiltration, and the accumulation of proinflammatory mediators at the site of inflammation were also significantly reduced in entospletinib-treated experimental mice compared to vehicle-treated animals.

Conclusion: Based on our results, pharmacological inhibition of Syk tyrosine kinase effectively reduces MSU crystal-induced neutrophil activation and gouty arthritis under experimental conditions, suggesting the potential for Syk inhibition as a therapeutic strategy for acute gout attacks in the future.

P-43 The effect of JAK inhibitors in experimental autoimmune skin blistering

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Introduction: Subepidermal autoimmune skin blistering diseases are characterized by autoantibody formation against components of the dermal-epidermal junction, leading to local proliferation and activation of inflammatory cells and consequent dissociation of the dermo-epidermal junction. Our previous experiments with gene-deficient mice have suggested the role of tyrosine kinase signalling pathways in the development of autoimmune skin blistering. Baricitinib is a small molecule JAK inhibitor registered for the treatment of several autoimmune diseases. The effect of baricitinib on the development of autoimmune blistering diseases is currently unknown.

Aims: We aimed to investigate the effect of systemic baricitinib treatment in a widely used animal model of autoantibody-induced skin blistering.

Methods: Wild-type mice were treated twice daily with 10 or 25 mg/kg baricitinib by oral gavage. Experimental autoimmune skin blistering was induced by subcutaneous injection of antibodies against type VII collagen (Col7). The extent and severity of the skin lesions and the appearance of erosions were then monitored for 14 days. Histological changes were assessed using H&E-stained skin biopsy samples. Leukocyte accumulation in the skin was measured by flow cytometry, proinflammatory mediator levels by ELISA. The in vitro effects of baricitinib on neutrophils were investigated in ROS production and human skin separation assays.

Results: Baricitinib treatment initiated concurrently with disease induction reduced both the extent and severity of skin involvement in a dose-dependent manner compared with vehicle-treated controls, with a reduction of up to 75% at the higher dose. When treatment was started after the appearance of the first skin lesions, baricitinib similarly resulted in a significant decrease in all evaluated parameters. Histological analysis demonstrated reduced dermo-epidermal separation in the treated group. Baricitinib therapy also led to decreased immune cell infiltration and lower levels of inflammatory mediators in the skin. Furthermore, baricitinib inhibited superoxide production by neutrophils in a dose-dependent manner.

Conclusion: Systemic baricitinib therapy inhibits the development of anti-Col7-induced skin blistering. This may be through its suppressive effects on inflammatory cell infiltration in the skin. Our results raise the possibility of the therapeutic use of baricitinib in autoimmune skin blistering diseases.

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P-44 A HoxB8-derived neutrophil model to study single gene function in inflammation and infection

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From the different possibilities to derive in vitro culturable, genetically tractable murine neutrophil precursors, the HoxB8 system has gained popularity over the years. Here we provide an extensive characterization of the *in vivo* behaviour of neutrophils derived from CRISPR-Cas edited HoxB8 progenitor cells.

Genetic manipulation of the HoxB8 progenitors is efficiently achieved via lentiviral transduction. Such progenitors readily differentiate to mature granulocytes upon in vivo adoptive transfer and display classic inflammatory neutrophil functions. We disrupted key neutrophil genes of migration, antibody-recognition and ROS-production to examine their role in immunocomplex-mediated sterile inflammation using the reverse passive Arthus reaction and serum-transfer arthritis models and found phenotypes consistent with the genetic manipulations performed on the progenitors. We also characterized the cells in an invasive Gram positive infection model obtaining results similarly corroborating the validity of the HoxB8 system to model neutrophil function.

In conclusion we carefully validated the effect of genetic manipulations in HoxB8 cells using several in vivo disease models comparing the phenotypes expected based on available literature to our findings. In light of our results it is safe to say that HoxB8-derived genetically modified neutrophil granulocytes recapitulate the phenotypes of their primary counterparts reasonably well. The HoxB8 model system thus provides reliable in vivo data on neutrophil biology and represents a potentially very time-efficient, streamlined way to generate and test any mutation including embryonic lethal ones in neutrophil-dependent disease models.

P-45 Dietary gliadin as a modulator of trigeminovascular sensitisation

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Keywords: gliadin, CGRP, trigeminovascular system

Gliadin, a major immunogenic component of gluten proteins found in wheat, has been implicated in a spectrum of gluten-related disorders beyond Celiac Disease. Non-Celiac Gluten Sensitivity (NCGS) is a prevalent condition affecting approximately 6–10% of the population, characterized by intestinal and extraintestinal symptoms in the absence of autoimmune enteropathy. Among these, headache – particularly migraine – is frequently reported. However, the underlying mechanisms linking NCGS to trigeminovascular activation remain poorly understood.

In this study, we investigated whether early-life dietary gliadin exposure influences trigeminal nociceptive pathways. Newborn Wistar rats received gliadin via oral gavage five times per week from postnatal day 2, while control animals received its vehicle. Following six weeks of treatment, blood samples were collected, and small intestinal segments were processed for histological analysis using hematoxylin-eosin staining. Calcitonin gene-related peptide (CGRP), a key neuropeptide in the pathophysiology of headache, was investigated to assess trigeminovascular function. CGRP release from meningeal afferents was measured in an *ex vivo* dura mater preparation following stimulation with capsaicin, a selective agonist of the transient receptor potential vanilloid type 1 (TRPV1) receptor. Quantification of CGRP levels in the collected samples was performed using ELISA method.

Gliadin-treated animals exhibited mild inflammatory alterations in the small intestine, accompanied by elevated plasma levels of the pro-inflammatory cytokine IL-6. Notably, CGRP release from trigeminal neurons demonstrated sex-dependent differences, both under basal conditions and following capsaicin-induced activation.

Our findings suggest that dietary gliadin may contribute to trigeminovascular sensitization, potentially through low-grade intestinal inflammation and systemic immune activation, with possible involvement of the gut–brain axis and sex-specific modulation.

This work is supported by the Albert Szent-Györgyi Research Grant.

YP-15 Effects of Methane Inhalation on Regional Blood–Brain Barrier Permeability and Brain Mitochondrial Function in a Rat Sepsis Model

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Keywords: Blood-Brain Barrier, Sepsis, Mitochondrial function

Introduction: Sepsis-associated encephalopathy (SAE) is closely linked to early disruption of the blood–brain barrier (BBB) and neuroinflammation, contributing to impaired cerebral function. Mitochondrial dysfunction is a key mechanism underlying sepsis-associated brain injury. We have demonstrated the anti-inflammatory properties of methane (CH_4), including preservation of mitochondrial function. This study aimed to investigate the effects of CH_4 inhalation administered at different time windows during sepsis progression on BBB permeability and brain mitochondrial function in an intra-abdominal sepsis model.

Methods: Adult male Sprague Dawley rats (n=68; 370 ± 30 g) were assigned to sham-operated (n=14), or septic (n=54) groups. Sepsis was induced via ip. injection of 5 mL/kg fecal inoculum. Septic rats were further divided into untreated (n=18) and CH_4-treated septic groups, the latter receiving 2.2% methane at defined time intervals (3–6 h, 21–24 h; n=18 each) during sepsis progression. Animal well-being was assessed using Rat-Specific Sickness Score (RSS) at 6, 16 and 24 h. At 24 h rats were anesthetized, Rat-specific Organ Failure Assessment (ROFA) scores, and mitochondrial respiration were measured in the cortex, cerebellum, hippocampus and striatum. In a second set of experiments, BBB permeability was examined regionally using Evans-blue dye.

Results: Sepsis markedly increased RSS and ROFA scores, significantly decreased mitochondrial respiration, and altered BBB integrity. CH_4 inhalation applied at 3–6 h of sepsis progression improved RSS scores but had modest effects on ROFA scores and no impact on BBB permeability. Treatment at 21–24 h significantly reduced ROFA levels and decreased BBB leakage across all examined brain regions. Early CH_4 treatment also had moderate effect on mitochondrial oxygen consumption, late phase CH_4 treatment also significantly increased complex II- and IV-linked oxidative phosphorylation in all examined brain regions. Mitochondrial membrane integrity was also preserved by CH_4 inhalation.

Conclusion: Methane inhalation demonstrates significant potential as an adjunctive treatment for sepsis and SAE. Our results highlight a time-dependent efficacy: early CH_4 administration provides modest clinical benefits, whereas late-stage intervention more effectively restores organ function and preserves BBB integrity. This suggest that CH_4 may be particularly effective in maintaining BBB function during the advanced stages of sepsis.

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YP-16 The effects of Time-restricted feeding on adipose tissue leukocytes

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Keywords: Time-restricted feeding, adipose tissue, leukocyte, mouse model, inflammation

The circadian clock coordinates immune and metabolic processes across tissues. In adipose tissue, immune cell composition is dynamically regulated and contributes to local inflammatory status. Feeding patterns, including time-restricted feeding (TRF), have been shown to modulate both circadian rhythms and inflammation, but their effects on adipose tissue leukocyte composition across the day remain incompletely understood.

We aimed to investigate how high-fat (HF) diet and TRF influence leukocyte composition in epididymal white adipose tissue (eWAT), with a particular focus on neutrophils and regulatory T cells (Tregs) at different circadian time points (ZT1 and ZT13).

Male wild-type C57BL/6 mice were assigned to four groups: normal chow *ad libitum*, high-fat diet *ad libitum*, or TRF with either diet limited to the first 10 hours of the active phase. After four weeks, tissues were collected. Leukocyte populations were analyzed by flow cytometry at ZT1 and ZT13, complemented by histological assessment.

HF diet and TRF both influenced adipose tissue morphology, with HF feeding promoting adipocyte hypertrophy, which was partially prevented by TRF. Both HF diet and TRF altered leukocyte composition in a time-dependent manner, indicating circadian modulation of immune cell distribution.. HF feeding increased neutrophil abundance and reduced the relative proportion of Tregs, whereas TRF shifted this balance toward a less inflammatory profile.

Our results indicate that HF diet and time-restricted feeding differentially shape adipose tissue immune cell composition in a circadian-dependent manner. The observed shifts in neutrophil and Treg populations may contribute to the modulation of inflammatory states in adipose tissue.

YP-17 Investigating the role of the Syk tyrosine-kinase in the pathogenesis of experimental systemic lupus erythematosus

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Keywords: SLE 1, Autoimmunity 2, Treatment 3

Systemic lupus erythematosus (SLE) is a lymphocyte-, phagocyte-mediated autoimmune disease. Since several patients do not respond to available therapies, there is a need for new therapeutic options. Previously, we have shown that the inhibition of Syk reduced the severity of an autoantibody-mediated experimental arthritis. Here, we aimed to set up an SLE model in our research group and to examine the role of the Syk tyrosine-kinase in the pathogenesis. Experimental SLE was induced by the intraperitoneal injection of pristane (PBS was used as control). Mice were treated with vehicle or the Syk-selective inhibitor entospletinib. Antinuclear antibody (ANA) screening was carried out by fluorescence microscopy. Peripheral blood leukocyte numbers were detected by flow cytometry. Arthritis was followed by clinical scoring and ankle thickness measurement. The presence of albuminuria and hematuria was tested by special strips and ELISA or microscopy.

Several months after the injection of pristane, mice became ANA-positive, while peripheral leukocyte (e.g. monocyte and B cell) numbers were elevated. We also observed a massive ankle thickening, albuminuria and hematuria in the pristane-treated group compared to control mice. Interestingly, entospletinib reduced joint inflammation and the albumin- and red blood cell-content of the urine samples.

We successfully set up the pristane-induced lupus model in our research group and found that selective Syk-inhibition resulted in a significant reduction of several disease manifestations. This means that entospletinib could be a possible drug candidate in the therapy of human patients with SLE in the future.

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YP-18 Adhesion-associated circadian reprogramming of circulating leukocytes with time-restricted food feeding

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Keywords: Time-restricted feeding, migration, leukocyte, circadian, adhesion

Leukocytes continuously circulate between blood and tissues in circadian manner. This trafficking depends on oscillations in adhesion molecules and migration capacity. Feeding patterns, including time-restricted feeding (TRF), have been shown to influence inflammatory responses, but their effects on circulating leukocyte migration dynamics remain incompletely understood.

We aimed to investigate the effect of TRF on the circadian dynamics of circulating leukocyte populations in chow and high-fat (HF) fed groups, with a particular focus on adhesion molecule profiles.

Male wild-type C57BL/6 mice were assigned to four groups: normal chow *ad libitum*, HF diet *ad libitum* or TRF with either diet limited to the first 10 hours of the active phase. After four weeks, peripheral blood samples were collected every 4 hours over a 24-hour period to assess circadian changes in circulating leukocyte counts. Leukocyte populations were analyzed by flow cytometry. In parallel, the expression of key adhesion molecules involved in leukocyte trafficking, such as CD62L, CD11a, CD11b, CD49d and PSGL-1, was assessed at two representative time points (day/night).

Total circulating leukocyte counts did not differ significantly between the groups, indicating that feeding interventions did not affect overall cell abundance. However, TRF markedly influenced the temporal dynamics of circulating immune cells in chow-fed animals, enhancing the amplitude of rhythmic oscillation of leukocyte counts. Both HF diet and TRF altered the expression of multiple adhesion molecules of circulating leukocytes, consistent with modified migratory capacity.

These findings suggest that feeding patterns primarily modulate immune cell migration dynamics, which may contribute to altered inflammatory responses.

YP-19 Effect of Syk inhibition on monosodium urate crystal-induced activation of human neutrophils

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Gouty arthritis is a common disease characterized by acute attacks, driven by an inflammatory process primarily mediated by phagocytic cells. This process is triggered by the deposition of monosodium urate (MSU) crystals in the joints and surrounding tissues. Previously, we demonstrated that genetic deficiency of the Syk tyrosine kinase significantly reduced MSU crystal–induced neutrophil responses and gouty inflammation in our experimental animal model.

In this study, I investigated the role of Syk tyrosine kinase in human neutrophils using a pharmacological approach, employing known Syk inhibitors currently undergoing clinical trials.

Human neutrophils were isolated from peripheral blood of healthy volunteers using density gradient centrifugation. Cells were pretreated with Syk inhibitors (entospletinib and lanraplenib) at various concentrations, or vehicle, and stimulated by MSU crystals. Reactive oxygen species production was measured by luminometry, LTB4 production by ELISA, p38 and ERK phosphorylation by Western blotting, and phagocytosis by flow cytometry.

MSU crystal–induced ROS production in neutrophils was effectively inhibited by both entospletinib and lanraplenib. Entospletinib effectively inhibited MSU-induced LTB4 production in neutrophils. Entospletinib (1 μM) and lanraplenib (10 μM) significantly reduced p38 and ERK phosphorylation compared to vehicle-treated samples. Neutrophils showed substantial phagocytosis of MSU crystals, this was reduced by approximately 50% with entospletinib and lanraplenib compared to vehicle-treated cells.

The Syk inhibitors used in this study significantly reduced MSU crystal–induced neutrophil responses. These findings, pending further detailed investigations, suggest that Syk inhibition may represent a potential therapeutic strategy for the treatment of acute gout attacks in the future.

YP-20 investigation of the role of dual oxidase-1 enzyme in urinary bladder overactivity

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Poster Session II. - Nervous system

P-46 Noradrenergic dysregulation in the triple-hit Lisket rat model of schizophrenia

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Keywords: schizophrenia, guanfacine, behavior

Schizophrenia is a multifactorial disorder with complex pathogenesis, including neurochemical disruptions. Aside from the classical dopaminergic hypothesis, the dysregulation of the noradrenergic system has been linked to its etiology. While α2-adrenergic receptor (α2-AR) agonists may improve positive symptoms; their effects on cognitive and negative symptoms remain inconsistent.

The goal of this study was to investigate the effects of guanfacine on schizophrenia-related behavioral phenotype and cerebral α2A-AR mRNA expression in the triple-hit Lisket rat model of schizophrenia, combining post-weaning social isolation, ketamine exposure and selective breeding in Long Evans rats.

Male Long Evans control (n=25) and Lisket model rats (n=26) were involved in the study, starting at 11 weeks of age. Intraperitoneal guanfacine (0.02, 0.1 or 0.5 mg/bwkg,) or its vehicle were administered daily for 11 days. behavioral phenotype was assessed using a reward-based Ambitus test before and during the treatment (8-11 days). Afterwards, region-specific cerebral α2A-AR mRNA expression was measured using RT-PCR.

The pretreatment Ambitus test revealed significant group differences: Lisket rats exhibited decreased exploration, motivational deficit, and impaired learning. Guanfacine dose-dependently altered exploration and cognition-related parameters, shaping an inverted-U pattern, particularly in Lisket animals. However, the drug did not affect the quantity of rewards collected, attention-related parameters, reference memory or overall data variability.

The α2A-AR mRNA expression was reduced in the prefrontal cortex, while increased in the brainstem, with a similar trend in the hippocampus in vehicle-treated Lisket rats compared to controls. Guanfacine exerted region-specific effects, resulting in decreased prefrontal and increased subcortical α2A-AR mRNA expression in both groups, with a kind of floor-effect in Lisket model rats at the cortical level.

These results provide insights into two aspects regarding the noradrenergic neuromodulation in schizophrenia: (1) α2A-AR mRNA expression is altered in a triple-hit rat model of the disorder, and (2) an α2A-AR agonist therapy alone is unable to improve either cognitive or negative symptoms. Furthermore, highlights the importance of integrative therapy, which can target neuromodulatory systems in multiple ways.

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P-47 Effects of pharmacological KAT II blockade on mitochondrial respiration in the central nervous system

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Keywords: tryptophan-kynurenine pathway, kynurenine aminotransferase II, kynurenic acid, mitochondria, respirometry

Introduction: Mitochondrial dysfunction is implicated in neurological and psychiatric disorders and may disrupt the tryptophan (Trp)-kynurenine (KYN) pathway, which produces bioactive metabolites, including the potentially neuroprotective metabolite kynurenic acid (KYNA). Our previous studies demonstrated region-specific reductions in cerebral mitochondrial respiration in mouse models lacking kynurenine aminotransferase (KAT) enzymes that produce KYNA. However, it remains unclear whether short-term pharmacological inhibition of these enzymes affects mitochondrial function. Here, we investigated the effects of inhibiting KAT II − the primary enzyme of brain KYNA synthesis − on mitochondrial O_2 consumption using the brain-penetrant irreversible inhibitor PF-04859989.

Materials and Methods: Male C57BL/6N mice (22–25 g; n = 6–7 per group) were used in this study. PF-04859989 was administered intraperitoneally (5 mg/kg) *in vivo* at 5, 24, and 48 hours prior to respirometry, while control animals received the vehicle alone. In separate experiments, cerebellar homogenates were incubated *ex vivo* with three concentrations of the inhibitor (5, 50, and 100 μM). Mitochondrial O_2 consumption was measured in cerebellar and hippocampal homogenates, including baseline respiration, complex I- and complex II-linked oxidative phosphorylation (CI- and CII-OXPHOS), and complex IV (CIV) activity, using high-resolution respirometry (Oroboros O2k).

Results: *In vivo* PF-04859989 treatment markedly reduced mitochondrial oxygen consumption, with CIV activity being decreased in the cerebellum in all groups. In addition, CI-linked OXPHOS was reduced at both 5 h and 24 h, whereas CII-linked OXPHOS declined at 24 h. By contrast, no changes were observed in the hippocampus following stimulation with exogenous substrates; only baseline respiration was decreased. *Ex vivo* incubation with the KAT II inhibitor suppressed baseline respiration, and CII-linked OXPHOS was consistently reduced at all tested concentrations, whereas CI-linked OXPHOS and CIV activity remained unchanged.

Conclusion: Acute pharmacological inhibition of KAT II markedly suppressed mitochondrial respiration both *in vivo* and *ex vivo*, indicating a rapid disruption of cerebellar energy metabolism. We hypothesize that *in vivo* effects could involve Trp-KYN pathway metabolites, whereas *ex vivo* reductions likely reflect a TCA cycle imbalance and decreased production of reducing equivalents for electron transport. The observed differences between brain regions suggest a region-specific sensitivity to KAT II inhibition.

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P-48 Cortical dynamics of associative learning in migraine: evidence from visual and audiovisual EEG tasks

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Keywords: migraine, EEG, associative learning

Cognitive deficits in migraine are not limited to ictal periods but can also be observed interictally. Previous behavioral findings suggest impaired performance in a visual acquired equivalence learning task, alongside preserved or even enhanced function in an audiovisual task. In the present study, we aimed to investigate whether cortical activity differs between migraine patients and matched controls during two different acquired equivalence tasks, and to characterize the role of cortical contributions in these learning processes.

64-channel EEG was recorded during a visual and an audiovisual acquired equivalence learning task in migraine patients and healthy controls. In the visual task, 17 migraine patients and 17 matched controls participated, while the audiovisual task included 18 patients and 18 controls. Stimulus onset-locked epochs were analyzed after preprocessing. Baseline-normalized power spectral density was estimated across canonical frequency bands. Time–frequency representations were computed, and task-related power changes were examined across predefined regions of interest. Group differences were assessed using cluster-based permutation statistics.

In the visual task, migraine patients showed significantly reduced beta-band activity compared to controls across a large portion of the scalp during the test phase in the post-stimulus time window. Time–frequency analysis confirmed reduced beta activity and revealed its temporal dynamics following stimulus onset. In contrast, the audiovisual task showed less pronounced group differences, with reduced activity in migraine patients during the test phase primarily localized to right frontal, temporal, and parieto-occipital regions.

The observed reduction in cortical activity in migraine patients during the visual task, especially in the test phase, aligns with previous behavioral findings and suggests that impaired performance may be associated with decreased cortical engagement. In the audiovisual task, the smaller differences in cortical activity between groups may reflect compensatory mechanisms, consistent with previously reported improved performance in migraine patients in audiovisual associative equivalence learning.

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P-49 Cortical correlates of stimulus properties during visual associative learning: a comparative EEG study

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Keywords: EEG, associative learning, stimulus properties

Visually guided acquired equivalence learning is influenced by the perceptual and semantic properties of visual stimuli. In a previous behavioral study, the modified Polygon task resulted in poorer acquisition performance than the original Rutgers Acquired Equivalence Test (RAET), whereas the performance in the test phase was not significantly affected. The two tasks have the same structure, but differ in stimulus properties: RAET uses visually complex and semantically richer stimuli, while Polygon applies simple grayscale geometric shapes with reduced semantic content and fewer supporting stimulus features. The aim of the present study was to investigate whether differences in learning performance are accompanied by differences in cortical activity.

64-channel EEG recordings were performed on 33 healthy participants while participants performed the tasks. Stimulus onset-locked and button press-locked (button press indicated the choice of the participants) epochs were analyzed. Task-related power changes were examined across frequency bands, and cluster-based permutation statistics were used to compare cortical activity between tasks.

EEG results revealed significant task related differences mainly during the acquisition phase of the tasks. In stimulus-locked epochs, theta-band differences were observed after stimulus onset over frontal/fronto-central and occipito-parietal regions, and during the test phase over occipital regions. Low-gamma activity also differed after stimulus onset during learning, involving frontotemporal and posterior clusters. In button press-locked epochs, theta-band differences appeared both before and after the response, involving posterior regions before the button press and a broader centro-parieto-frontal network after the response. Gamma-band differences were present before the response over frontotemporal and posterior regions, while gamma differences after the response, which may be related to reward processing, were mainly localized to left frontotemporal cortex.

These findings suggest that stimulus properties modulate cortical oscillatory activity during visual acquired equivalence learning. Stimuli with higher feature complexity and semantic content are associated with a distinct cortical oscillatory profile, especially in theta and gamma frequency ranges. The results indicate that stimuli with reduced semantic content and feature complexity may alter cortical processing primarily during acquisition, highlighting the importance of stimulus properties not only for learning performance but also for cortical activity in associative learning.

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P-50 Same performance, different dynamics: task effects in Spaceflight Learning

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Keywords: acquired equivalence learning, cognitive performance, spaceflight

Acquired equivalence paradigms assess acquisition, retrieval, and generalization within a unified framework. The Space Acquired Equivalence Test (SAET) extends the Rutgers version (RAET) by adding visual–spatial demands through rotated stimuli, potentially relevant in microgravity.

Forty control participants completed both tasks once, while one astronaut and two reserve astronauts completed 21 sessions in SAET and RAET across preflight, inflight, and postflight phases. Error ratios and reaction times were analyzed. Performance relative to controls was expressed as task-specific z-scores. Analyses included between-task comparisons in controls, astronaut vs. control comparisons, within-astronaut paired tests, and linear models examining session, phase, and task effects with FDR correction.

In controls, no differences were observed between SAET and RAET (all FDR-corrected $p \geq 0.92$), indicating comparable baseline performance. The astronaut and reserve astronauts outperformed controls across measures (all $p < 0.001$). In the astronaut, raw performance did not differ between tasks (retrieval reaction time (RRT): $p = 0.60$; generalization reaction time (GRT): $p = 0.50$). However, standardized comparisons revealed significant differences (RRT: $p = 2.52 \times 10^{-4}$; GRT: $p = 0.0021$, FDR-corrected), indicating task differences relative to the controls rather than absolute performance. Longitudinal analyses supported this pattern (RRT: $p = 0.0021$; GRT: $p = 0.0099$, respectively). In reserve astronauts, standardized comparisons showed task differences, but these were not confirmed by longitudinal models, and raw performance did not differ.

The astronauts outperformed controls across all measures, while SAET and RAET were comparable in controls and in raw performance. However, task differences emerged relative to controls and across time, particularly for retrieval and generalization, suggesting taskdependent longitudinal effects, though limited by the single-subject design.

P-51 Home cage gamma oscillaon patern in the rat prelimbic area predicts behavior in the modified successive alley paradigm

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The role of gamma oscillaons in regulang fear behavior has been widely implicated. Moreover, sub-ranges in this oscillatory range play disnct role in regulang fear behavior in fear condioning paradigms. Excessive innate fear and behavioral avoidance are core features of anxiety disorders. Therefore, animal models, monitored in more naturalisc environments over longer periods of me, are of great translaonal value. Beyond basolateral amygdala (BLA), prefrontal cortex (PFC) oscillaon and their funconal connecvity are crucial in regulang fear behavior. However, whether PFC and BLA gamma oscillaon paterns can predict innate fear behavior and voluntary risk taking, is sll elusive.

We used the modified successive alley paradigm and invesgated if gamma oscillatory patern has a predicve power upon alley behavior. We recorded LFP acvity in the home cage and when animals could enter the aversive alley. We calculated the rao of high (70-95) Hz and low (40-70 Hz) gamma power range. We checked also if PFC BLA funconal connecvity is predicve on behavior in these oscillatory ranges.

We found that a) the modified paradigm can separate innate higher and lower fear level between trials. b) Only PFC home cage oscillatory paterns can predict fear level under later aversive condions. c) The gamma subrange compeon emerges at the level of funconal connecvity between PFC and BLA. d) A PFC lead funcon in the high gamma subrange predicts behavioral performance.

We conclude that PFC gamma oscillaons may represent a potenal target for intervenons aimed at modulang elevated innate fear and anxiety.

P-52 Sex-dependent effects of kisspeptin-13 on core temperature in rats: a telemetry study

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Kisspeptins are master regulators of the reproductive axis that have also been implicated in the sex-dependent modulation of metabolism and body temperature. Our aim was to investigate how intraperitoneally or intranasally administered kisspeptin-13 (Kp-13) regulates core temperature and locomotor activity in male and female Wistar-Hannover rats.

3-month-old rats were implanted with an E-mitter and kept in a 12-12 h light-dark cycle with the lights on from 6 pm. After one week of recovery, the core temperature and activity were recorded for two days using a telemetry system. In the intraperitoneal (ip.) protocol, the animals received 0.2 ml PBS on the first day, and 13 or 26 µg of Kp-13 on the second day. In the intranasal (in.) protocol, 20 µl PBS was delivered on day 1, followed by 1 or 10 µg of Kp-13 on day 2. The animals were treated around 8:30 am on both days, followed by 24 h of telemetry.

Kp-13 modulated body temperature in a sex-dependent manner. In males, both doses of ip. Kp-13 induced a long-lasting hyperthermia affecting both the dark and light phases. In females, however, the larger dose of ip. Kp-13 had a marked hypothermic effect in the light phase. The same effect was observed in females after intranasal delivery. Locomotor activity was not altered significantly by Kp-13 in any of the treatment protocols.

The sex-dependent effect of Kp-13 is in accordance with the literature and may result from the sexual dimorphism of kisspeptin signaling or indirect effects mediated by gonadal steroids.

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P-53 CB1R blockade improves force generation and motor coordination without interference with calcium signaling in mdx mice

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Keywords: skeletal muscle, endocannabinoid system, CB1 receptor, Duchenne muscular dystrophy, calcium signallingDuchenne muscular dystrophy (DMD) is an X-linked disorder caused by dystrophin deficiency, leading to sarcolemmal instability, elevated intracellular Ca^{2+} concentrations, and progressive muscle degeneration. Increased Ca^{2+} drives mitochondrial dysfunction and oxidative stress, key contributors to disease pathology. The ECS comprises cannabinoid receptors (CB1R, CB2R), endogenous ligands, and associated metabolic enzymes. CB1R is expressed in skeletal muscle and modulates mitochondrial metabolism and myogenic processes. Recent evidence suggests that CB1R signaling (over activity) may worsen dystrophic phenotype. Pharmacological inhibition of CB1Rs, with the inverse agonist rimonabant has been shown to delay the disease onset, to improve regeneration and reduce inflammation.

Here, 12-weeks-old control and mdx mice were administered rimonabant for two weeks. Following this chronic treatment functional assessment revealed a mild rescue in grip strength, and a significant improvement in rotarod performance in mdx mice compared to control, indicating improved motor performance and coordination. In isolated flexor digitorum brevis (FDB) fibers, excitation–contraction coupling was mainly preserved, with no change in voltage-dependent Ca^{2+} release. We also evaluated the effect of acute rimonabant treatment on the Ca^{2+} homeostasis of FDB fibers and the results align with the findings of the chronic application.

These results suggest that CB1R signaling modulate muscle force development independently of excitation–contraction coupling, contributing to functional muscle improvement. Targeting ECS signaling may therefore represent promising modifier pathway, but not a root cause cure for DMD.

P-54 A novel lumen–neuron axis in irritable bowel syndrome

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Keywords: irritable bowel syndrome, enteric nervous system, proteases

Irritable bowel syndrome (IBS) affects approximately 12% of the population. Besides central components, factors acting within the intestinal lumen and the role of the enteric nervous system have also been implicated in its pathogenesis. We previously demonstrated that fecal supernatants from patients with diarrhea-predominant IBS (IBS-D) exhibit increased serine protease activity, which may induce enhanced intestinal permeability and visceral pain through cleavage of protease-activated receptors (PARs). The aim of our study was to investigate how fecal supernatants prepared from patients with IBS-D and constipation-predominant IBS (IBS-C) affect the activity of enteric neurons, with a focus on proteases.

Fecal supernatants from IBS-D and IBS-C patients and healthy controls were applied to submucous plexus neurons isolated from guinea pig colon, while action potentials were recorded. Fecal supernatants from IBS-D and IBS-C patients induced significantly stronger neuronal activation compared with those from healthy controls. Both the serine protease inhibitor (FUT-175) and the cysteine protease inhibitor (E-64) significantly reduced the activating effect of fecal supernatants from IBS-D on enteric neurons, but had no effect on fecal supernatants from IBS-C patients. The same pattern was observed with the PAR-1 antagonist (SCH79797).

These findings reveal a previously unrecognized lumen–neuron communication axis in IBS and indicate fundamentally different pathomechanisms between IBS-D and IBS-C. IBS-D is characterized by protease- and PAR-1-dependent neuronal activation, whereas in IBS-C, mediators appear to act through protease-independent pathways. These results establish a functional link between fecal protease activity and enteric nervous system signaling, offering new therapeutic opportunities for subtype-specific treatment of IBS.

P-55 Age-related changes in respiratory system mechanics and lung morphometry

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Aging leads to degenerative changes in lung structural and mechanical properties that may impact breathing. Relatively little is known about how age-related alterations in lung mechanical properties impact expiratory duration (T_E) and residual volume (RV).

The present study tested three hypotheses: 1) alveolar airspace increases with age due to alveolar wall breakdown, 2) lung elastic recoil decreases, slowing expiratory flow (E-flow), and 3) airway compliance (Caw) decreases, promoting air trapping reflected by increased RV.

To test these hypotheses, we evaluated changes in alveolar size using histological assessments, mechanical properties using forced oscillation perturbations with a programmable mechanical ventilator and breathing parameters using plethysmography in young (6 months) and old (24 months) Fischer 344 rats.

Mean linear intercept (L_m) was higher in old (65 ± 10 μm) compared to young (55 ± 5 μm) rats, indicating alveolar airspace increase. In old rats, decreased elastic recoil was indicated by increased parameter K (0.178 ± 0.010 vs. 0.167 ± 0.013 in young) and higher V_{10}/TLC (80.7 ± 1.2% vs. 78.5 ± 2.5% in young). In addition, RV was higher in old rats (0.41 ± 0.10 mL vs. 0.27 ± 0.05 mL in young), consistent with air trapping. These mechanical changes were associated with increased E-flow (~30%) in old rats, attributable to increased tidal volume (2.08 ± 0.41 mL vs. 1.53 ± 0.36 mL in young) as T_E was unchanged.

These results indicate important changes in the lung parenchyma, causing alteration in passive and active properties of the respiratory system in aging rats.

YP-21 Dose-dependent effects of long-term ciprofloxacin treatment on brain mitochondrial function and neurobehavioral performance in rats

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Keywords: mitochondrial dysfunction, ciprofloxacin, behavior, blood-brain barrier, high-resolution respirometry

Fluoroquinolone antibiotics - including ciprofloxacin (CPX) - are widely prescribed for their broad-spectrum antimicrobial activity. However, accumulating evidence indicates that these medications can have adverse effects on the central nervous system (CNS), including anxiety, depression, and cognitive impairment. One potential contributing factor is the ability of CPX to cross the blood–brain barrier (BBB), thereby influencing the function of neurons and glial cells. Despite these findings, the dose-dependent effects of CPX on mitochondrial function in the CNS, as well as their relationship to behavioral changes, have not yet been sufficiently clarified. Therefore, the present preliminary study aims to investigate the effects of long-term oral administration of CPX administration on mitochondrial respiration and neurobehavioral performance in an experimental rat model.

Adult male rats (n=10) received CPX gavage for 28 consecutive days at doses of 10, 50, or 100 mg/ml twice daily. Neurobehavioral changes were evaluated using Open Field (OF) and Elevated Plus Maze (EPM) tests to assess locomotor activity and anxiety-like behavior. Mitochondrial respiration was analyzed in the periphery (kidney) and CNS (brain tissue samples) by high-resolution respirometry (HRR), focusing on electron transport chain Complex I and II activity, oxidative phosphorylation capacity, LEAK respiration and respiratory control ratio (n = 4-4).

Results: the highest CPX dose was accompanied by significant anxiety-like behavior and a marked reduction in spontaneous exploratory activity as demonstrated by EPM and OF tests, respectively. Based on the results obtained from the currently available samples, mitochondrial respiration in renal tissue was already impaired in the 10 mg/mL group, particularly at the level of complexes I–II, but complex IV was also affected. HRR data suggest that CPX treatment also alters mitochondrial respiration in the central nervous system, since, for example, a significant decrease in the capacity of complex IV can be observed in the hippocampus, regardless of the CPX dose administered.

These findings suggest that long-term CPX exposure may cause mitochondrial dysfunction in the CNS, which may contribute to the observed behavioral alterations. This study provides a first insight into the possible mechanisms underlying CPX-induced CNS dysfunction.