# HELLENIC SOCIETY OF PHYSIOLOGY CONFERENCE

MARCH 12-14 2026

## AUDITORIUM HIPPOCRATES

FACULTY OF MEDICINE SCHOOL OF HEALTH SCIENCES UNIVERSITY OF THESSALY LARISSA

# HELLENIC SOCIETY OF PHYSIOLOGY CONFERENCE 2026

# HIPPOCRATES AUDITORIUM, LARISA, 12-14 MARCH 2026

## Welcome Address by the President of the Hellenic Society of Physiology

Dear fellow colleagues,

It is with great honor and sincere joy that we welcome you to the Hellenic Society of Physiology Conference 2026. This gathering is dedicated to advancing Physiology - a cornerstone discipline for understanding the function of living organisms and the nature of life itself.

Physiology stands at the foundation of normality and health. As eloquently articulated by the Professor, Physician, and Philosopher Georges Canguilhem, *"Health is not merely defined by conformity to a statistical norm, but by the organism's capacity to establish new norms, adapt and respond".* Physiology is therefore inherently dynamic, continually refining our understanding of regulation, resilience, and disease. It forms a vital bridge between basic biomedical research and clinical practice, underpinning translational science and the enduring pursuit of "bench to bedside," whereby mechanistic insight is transformed into diagnostic and therapeutic progress.

The Hellenic Society of Physiology, with its longstanding academic contribution to Greek biomedical science, is committed to fostering rigorous scientific dialogue and collaborative excellence. Our vision is to further open the Society to young investigators and distinguished scholars alike, while engaging established leaders in shaping its future, cultivating an inclusive environment. It is essential that our members align their efforts toward advancing and responsibly serving the field. As the Board of Directors, we are dedicated to fostering interdisciplinary dialogue, mentorship, and scientific synergy that strengthens national and international engagement.

The scientific program has been carefully designed to present cutting-edge advances while inspiring students and early-career scientists to participate actively in scholarly exchange, reaffirming the integrative and multidisciplinary essence of Physiology.

We sincerely thank all contributors and participants whose presence reinforces our collective commitment to the continued advancement of Physiology. May this Conference stimulate reflection, constructive collaboration, and renewed dedication to the pivotal role of Physiology in contemporary science and medicine.

**Sincerely,**

Mara Simopoulou
Professor of Physiology-Clinical Embryology
Medical School
National and Kapodistrian University of Athens, Greece
President of the Hellenic Society of Physiology

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# ORAL PRESENTATIONS

**Thursday March 12th, 2026**

**16:30 - 17:30 Auditorium Hippocrates**

**OP1-OP5 Chairs**

**Asst. Prof. Yannis Simos (UoI), Prof. Maria Simopoulou (NKUA), Prof. Michael Koutsilieris (NKUA)**

**Friday March 13th, 2026**

**8:30 - 9:30 Auditorium Hippocrates**

**OP6-OP10 Chairs**

**Asst. Prof. Antonia Marazioti (UoPel), Asst. Prof. Panagiotis Sapountzis (UoC), Asst. Prof. Konstantinos Tsamis (UoI)**

**Saturday March 14th, 2026**

**9:30 - 10:30 Auditorium Hippocrates**

**OP11-OP15 Chairs**

**Asst. Prof. Konstantinos Palikaras (UoC), Asst. Prof. Myrto Denaxa, Prof. Constantinos Mikelis (UoP)**

**OP1. Differential gene expression between first-time chronic endometritis (CE), persistent Ce and cured CE patients.**

*D. Tsagkaraki[1], K. Sfakianoudis[2], E. Maziotis[1], A. Trypidi[1], M. Androutsopoulou[1], A.Toska[1], A. Pantou[2], S. Grigoriadis[1], M. Chronopoulou[2], S. Pousias[2], P. Tzonis[2], M.Simopoulou[1], K. Pantos[2]*

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Chronic endometritis (CE), characterized by persistent inflammation of the endometrial tissue, is most commonly caused by bacterial infections of reproductive tract. CE affects endometrial receptivity and has been correlated with higher risk for infertility. Treatment of CE employing antibiotics has been suggested to be partially effective. Ineffective treatment leads to persistent inflammation. Molecular data regarding differential gene expression between first-time CE, persistent CE and cured CE patients are lacking. A total of 178 women were enrolled in this prospective study, conducted between04/2025-12/2025. 57 participants had a first-time diagnosis of CE(first-time CE-group), CE was effectively treated in 29 participants (cured CE-group) while, 33 women presented with persistent CE following antibiotic treatment (persistent CE-group). A total of 59women, without CE diagnosis, were served as the control group. CE diagnosis was histologically confirmed via CD-138. Endometrial tissues were analyzed for the expression of five immune-related genes by qPCR. The expression levels of JCHAIN, IGHG4, required for the function of immunoglobulins, NFkB1 and IFNg, which promote proinflammatory expression, TVP23A, highly expressed in macrophages and GAPDH, serving as the reference gene, were evaluated. No significant difference was observed regarding IFNg (p=0.33) and TVP23A(p=0.27) expression, while levels of IGHG4(p=0.007), JCHAIN (p=0.009) and NFkB1(p=0.049) were significantly different among the groups. IGHG4 expression was significantly increased in both first-time CE-group (ΔCt:10.32±7.13; fold=5.87) and persistent CE-group (ΔCt:7.85±4.88; fold=32.61) compared with control group (ΔCt: 12.87±6.18). Also, IGHG4levels were higher in persistent CE-group (ΔCt:7.85±4.88; fold=32.61) compared to cured CE-group (ΔCt:12.23±6.24; fold=1.56). First-time CE-group presented with significantly higher JCHAIN expression levels (ΔCt:6.85±2.11; fold=2.24) compared to control (ΔCt:8.02±2.01) and to cured CE-group (ΔCt:8.37±2.25; fold=0.78). Regarding NFkB1, first-time CE-group appeared with significantly lower levels (ΔCt:4.82±1.23; fold=0.71) compared to control (ΔCt: 4.32±1.15). Gene expression following successful CE treatment is similar to the control group. IGHG4is overexpressed in persistent CE cases, while JCHAIN is overexpressed mainly in first-time CE patients.

**OP2. Hypoxia-dependent induction of AGPAT4 stimulates lipid droplet accumulation and migration of cancer cells.**

*Zoi Kakae[1], Angeliki Karagiota[1,2] and Effrosyni Paraskeva[1]*

[1]Laboratory of Physiology and [2]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece

Cancer cells reprogram lipid metabolism to overcome the challenges of the hypoxic tumor microenvironment (TME). Adaptation to hypoxia is largely mediated via activation of transcription by the Hypoxia Inducible Factors (HIFs). HIF-1 upregulates, among others, the expression of AGPAT2 (Acylglycerolphosphate acyltransferase 2) in the triacylglyceride (TAG) biosynthesis pathway. AGPAT2 belongs to a family of five enzymes, that catalyze the synthesis of phosphatidic acid (PA), a TAG intermediate, signaling molecule and precursor of phospholipids. Although AGPATs catalyze the same reaction, they have unique non-overlapping roles. Having shown that induction of AGPAT2, increases lipid accumulation, proliferation and chemoresistance of cancer cells under hypoxia (1), we have now analyzed the expression of all AGPAT isoforms. Our findings demonstrate that the expression of AGPAT4, although barely detectable under physiological O2 conditions, is highly upregulated under hypoxia (1% O2) in different cancer cell lines. Bioinformatic analysis of publicly available cancer patient data shows that the expression of AGPAT4 is positively correlated with the expression of both HIF1Aand a hypoxia gene signature in human cancers. The induction of AGPAT4 is also observed upon stabilization of HIF-1α by the prolylhydroxylase inhibitor DMOG and is prevented by siRNA mediated knockdown of HIF-1α in HeLa cells. In addition, the expression of SOX9, a factor recently shown to mediate transcription of AGPAT4 in cancer cells, is also increased under hypoxia, indicating the operation of a HIF-1/SOX9/AGPAT4 axis in the hypoxic TME. Importantly, the expression of AGPAT4 under hypoxia is required for the accumulation of lipid droplets and cell migration, two features associated with cancer cell aggressiveness. Accordingly, in several cancer types highAGPAT4 expression is associated with negative prognosis, highlighting its importance for human patient tumor growth.

1. Triantafyllou et al. Biochim Biophys Acta Mol Cell Biol Lipids. 2018;1863(9):1142-1152.

**OP3. Neuroprotective and Anti- Inflammatory Effects of Anthocyanins.**

*Nikoletta Christoudia[1*], Charalampos Mpakas[2*], Elpiniki Apostolidou[2*], Afroditi Kapourani[3], Maria Koromili[3], Spyros Pettas[1,2], Spyros Didos[4], Panagiotis Barmpalexis[3], Chryssa Bekiari[5], Eirini Kanata[2], Anagnostis Argyriou[4,6], Konstantinos Xanthopoulos[2], Theodoros Sklaviadis[2], Dimitra Dafou§[1]*

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*Equal first author

Neurodegenerative disorders (NDs) are characterized by progressive neuronal loss, neuroinflammation, and increased oxidative stress, which are closely associated with disease onset and progression and pose major challenges for prevention and therapy. Anthocyanins (ACNs) are natural, water-soluble vacuolar pigments, and are considered safe bioactive compounds that can be readily obtained through dietary intake. ACNs are known potent antioxidants with anti-inflammatory properties. We developed and characterized ACN-rich polyphenol extracts, utilizing a GRAS (Generally Recognized asSafe) industrial-scale ACN extraction process from dried grape skins. ACN extracts were formulated with β-cyclodextrin and were administered through drinking water in daily doses ranging between 30 and 200mg/kg for 30 days, without any toxic effects (as evidenced by clinical evaluation, body weight monitoring and histopathological examination). In addition, gut microbiome analysis did not indicate disturbances of the normal gut microbiome in mice received the formulated ACNs. A medium dose(150mg/kg), administered without β-cyclodextrin formulation, was then evaluated as a protective agent in the murine Experimental Autoimmune Encephalomyelitis (EAE), a widely used model of autoimmune inflammatory diseases of the central nervous system (CNS) that recapitulates many pathological features of Multiple Sclerosis (MS). ACN-treated mice exhibited significantly reduced demyelination in white matter (Klüver–Barrera staining) compared to controls with delayed

onset and progression of clinical symptoms and downregulation of pro-inflammatory cytokines. Analysis of secondary metabolites revealed modulation on host tryptophan metabolism, increasing the production of neuroprotective metabolites such as kynurenic acid, further supporting additional research on metabolomic studies. Collectively, these findings highlight the therapeutic potential of anthocyanins as neuroprotective agents capable of mitigating neuroinflammation supporting dietary anthocyanin supplementation as a promising strategy for preventing or delaying the progression of neurodegenerative diseases.

**OP4. The role of ROCK-CREB pathway on mutant KRAS-induced malignant pleural effusion formation.**

*Spyros Artemis[1], Andriana Plevriti[1], Isidora Liepouri[1], Stavros Sideris[2], Constantinos M. Mikelis[1], Antonia Marazioti[2]**

[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, School of Health Sciences, University of Patras

[2]Basic Sciences Laboratory, Department of Physiotherapy, School of Health Sciences, University of the Peloponnese

Malignant pleural effusion (MPE) represents an end-stage manifestation of metastatic cancer, with limited therapeutic options. Mutant KRAS-bearing tumor cells disseminate into the pleural cavity, driving disease formation. The presence of KRAS mutations in tumors is usually linked with treatment resistance. Emerging evidence implicates the Rho-kinase (ROCK)-CREB signaling pathway in invasion and proliferation of KRAS-mutant tumors, placing them as possible KRAS downstream targets for MPE treatment. This study investigated the effects of the ROCK inhibitor Fasudil in two KRAS-mutant cell lines and in an animal model of mutant KRAS-induced MPE. Murine syngeneic Lewis lung carcinoma (LLC) and pleural mesothelioma (KPM1) cells were treated with varying concentrations of Fasudil and phosphorylated CREB protein levels, cell proliferation and colony formation on soft agar were examined. ROCK inhibition via Fasudil treatment significantly reduced proliferation and soft-agar colony formation. Mechanistically, Fasudil suppressed CREB phosphorylation, that is constitutively highly induced in both KRAS-mutant cancer cell lines. In the in vivo setting, C57BL/6 mice were intrapleurally injected with LLC cells to induce MPE. Upon initial tumor formation, intraperitoneal Fasudil administration attenuated pleural tumor growth and prevented MPE development. These findings demonstrate that Fasudil suppresses KRAS-mutant tumor progression in vitro and in vivo via ROCK- CREB inhibition, suggesting its potential as a novel therapeutic approach of MPE. Acknowledgement This work was supported in part by the Hellenic Foundation for Research and Innovation (00376) and by MEDIKOS Award from the University of Patras. Andriana Plevriti is Supported by “Andreas Mentzelopoulos Foundation” Fellowship.

**OP5. The effect of intraovarian platelet-rich plasma (PRP) injection on the ovarian function andin vitro fertilization (IVF) outcomes of poor ovarian response (POR) patients.**

*M. Androutsopoulou[1], A. Pantou[2], K. Pantos[2], E. Maziotis[1], A. Trypidi[1], S. Grigoriadis[1],G. Papadogkonas[1], D. Charitaki[1], D. Tsagkaraki[1], A. Pantou[2], H. Pappa[2], C. Markomichali[2], D. Kappou[2], K. Dafopoulos[3], K. Sfakianoudis[2], M. Simopoulou[1]*

[1]Unit of Reproductive Physiology-Clinical Embryology, Department of Physiology-Medical School-National and Kapodistrian University of Athens, Athens, Greece

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Poor ovarian response remains a major challenge in medically assisted reproduction and is associated with both advanced maternal age and ovarian dysfunction. Platelet-Rich-Plasma has the potential to enhance ovarian function and promote folliculogenesis by stimulating angiogenesis of the ovarian microenvironment. The aim of this study is to assess the effect of intraovarian PRP injection on the ovarian function and IVF outcomes of women over 40 with a diagnosis of POR. This single-center prospective cohort study included 144 nulliparous women aged ≥40diagnosed with POR, according to the Bologna criteria, undergoing IVF. Women presenting with reproductive, endocrine, autoimmune and genetic disorders and BMI 30were excluded. Participants were allocated into two groups: one receiving intraovarian PRP injections prior to controlled ovarian stimulation (COS)(n=72) and a control group subjected to COS(n=72).No statistically significant difference was observed between the two groups prior to study enrollment, regarding AMH, FSH, LH, E2 on trigger day, AFC, number of oocytes collected and number of obtained embryos from their previous attempt. Similarly, no significant differences in female age (43.82±3.63 vs 43.23±4.52, p=0.48) and male partner age were observed. The PRP group demonstrated significantly higher AMH levels(0.76±0.47 vs 0.52±0.32,p=0.01), AFC(3.57±2.31 vs 2.85±1.81,p=0.04), number of oocytes retrieved(2.89±2.64 vs 1.93±1.36, p=0.001) and MII oocytes(2.26±2.25 vs1.39±1.08,p=0.005) compared to controls. Furthermore, rates of two-pronuclei zygotes (p=0.07), cleavage-stage embryos(p=0.11) and clinical pregnancy(p=0.47) did not differ significantly, while no embryos were available for transfer in 22 PRP and 26 control cases. Finally, following embryo transfer, more patients in the PRP group presented with remaining cryopreserved embryos (11/72 vs 3/72,p=0.03). This study underscores a positive clinical effect of intraovarian PRP injection in POR women of advanced reproductive age. Interestingly, intraovarian PRP enhances AFC

and AMH levels, increases the number of oocytes and cryopreserved embryos, ameliorating the chances for cryopreservation.

**OP6. Study of the role of the soluble CD163 (sCD163) on endothelial physiology.**

*Andriana Plevriti[1], Maria Giannakopoulou[1], Md. Sanaullah Sajib[2], Margarita Lamprou[1], Eleni Mourkogianni[1], Georgios Sivvas[1], Elias Liolis[1], Constantinos M. Mikelis[1,2*]*

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CD163 is a scavenger receptor primarily expressed by monocytes/macrophages, and its soluble form (sCD163) is recognized as an inflammatory biomarker. Increasing evidence suggests that sCD163 may act as a paracrine signaling molecule influencing endothelial function and vascular remodeling, key processes associated with metastatic dissemination. However, the direct effects of sCD163 remain poorly defined. In this study, we identified that sCD163 is secreted by human triple negative breast cancer cells, and its secretion is proportional to their metastatic potential. We further investigated its impact on endothelial physiology in vitro and in vivo with a particular focus on cytoskeletal regulation and barrier function. sCD163 significantly promoted the migration of human umbilical vein endothelial cells (HUVECs) and murine lung microvascular endothelial cells (LMVECs), with a small effect on proliferation and no impact on tube formation. In parallel, sCD163 impaired endothelial barrier integrity, as evidenced by decreased trans-endothelial electrical resistance (TEER). To study the molecular cues of sCD163-inducedcellular activities, we focused on the role of the small GTPase RhoA, an important mediator of cytoskeleton-dependent cellular functions. sCD163 induced RhoA and myosin light chain activation, while pharmacological inhibition of the RhoA/ROCK axis significantly attenuated sCD163-induced migration and restored endothelial barrier function. Moreover, RhoA silencing in HUVECs and LMVECs from mice with endothelial-specific RhoA deficiency abolished the migratory response to sCD163, confirming a central role for the RhoA signaling pathway. Finally, in mice with endothelial-specific RhoA deficiency sCD163-induced permeability was blocked, confirming the involvement of endothelial RhoA in sCD163-mediated vascular responses in vivo. Collectively, these findings demonstrate that sCD163 modulates endothelial migration and barrier integrity through activation of the RhoA/ROCK pathway, highlighting a previously unrecognized role of sCD163 in the physiology of vascular-related conditions.

**OP7. Anticancer action of quercetin and myricetin on glioblastoma cells: the potential role of CD71.**

*Georgios Markopoulos, Christina Tsialiou, Vivia Horaj, Lampros Lakkas, Kostas Tsamis, Dimitrios Peschos, Yannis Simos*

Laboratory of Physiology, Faculty of Medicine, University of Ioannina, 45110 Ioannina, Greece

Background Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with limited therapeutic options. Natural polyphenols such as quercetin and myricetin exhibit anticancer and anti-inflammatory properties and modulate pathways relevant to glioma proliferation and survival [1,2]. CD71 (transferrin receptor 1), a key regulator of iron uptake and cellular metabolism, is frequently associated with tumor growth and may represent a relevant mechanistic link [3].

Methods Human glioblastoma U87 cells were treated with increasing concentrations of quercetin and myricetin. Cell viability was assessed by MTT assay and $IC_{50}$ values were estimated. Cell cycle distribution was analyzed by flow cytometry following propidium iodide staining. Ongoing experiments include assessment of intracellular reactive oxygen species (ROS), apoptosis analysis using Annexin V/PI staining, and evaluation of CD71 surface expression by flow cytometry.

Results Both compounds reduced U87 cell viability in a dose-dependent manner, with estimated $IC_{50}$ values of ~600 μM for quercetin and ~400 μM for myricetin. Cell cycle analysis demonstrated G1 phase arrest at lower concentrations and a marked increase in the sub-G1 population at higher concentrations, consistent with apoptotic cell death. Ongoing analyses aim to determine whether these effects are associated with oxidative stress induction, apoptotic signaling, and modulation of CD71 expression.

Conclusions Quercetin and myricetin exert antiproliferative and cell cycle-modulating effects in glioblastoma cells. Ongoing functional and phenotypic analyses focusing on ROS generation, apoptosis, and CD71 expression are expected to further elucidate the underlying mechanisms, supporting a potential role for CD71-associated pathways in GBM response to natural polyphenols [1–3].

References 1. Markopoulos et al. Cancers (Basel), 2025; 17(24):3922. DOI:10.3390/cancers17243922

2. Vartholomatos et al. Biomedicines, 2022; 10(5):935. DOI:10.3390/biomedicines10050935

3. Markopoulos et al. Journal of Clinical Medicine, 2025; 14(23):8265. DOI:10.3390/jcm14238265

**OP8. Tau protein as a regulator of mitochondrial function and dynamics**

*Eleni Tsakiri[1], Carlos Campos-Marques[2,3], Ildete Luísa Ferreira[4,10], Christina Ploumi[1], Antonis Roussos[1], Eirini Mytilinaiou[1], Chrysoula Dioli[2,3,6], Martina Samiotaki[7], Anastasia Vamvaka Iakovou[6], Kalliopi Skourti[6], Clarissa Waites[8,9], Nuno Sousa[11,12], Joana M Silva[2,3], A. Cristina Rego[4,5], Ioannis Sotiropoulos[2,3,6*] and KonstantinosPalikaras[1*]*

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Mitochondrial damage is a shared hallmark of brain aging and neurodegeneration. While pathological Tau mutations are known to impair mitochondrial dynamics and function, the physiological role of wild-type Tau in the maintenance of mitochondrial homeostasis remains poorly understood. Here, using C. elegans and mice lacking PTL-1, the nematode Tau-like homolog, and Tau respectively, we demonstrate that Tau deficiency enhances mitochondrial respiration, ATP production and mitophagy, promoting a pro-fusion mitochondrial network in neurons. Tau-deficient nematodes also display increased resistance to stressors, including heat and mitochondrial insults. Strikingly, loss of FZO-1,the mitofusin homolog, abolishes these effects, reducing mitochondrial function and stress resistance in PTL-1 deficient nematodes. Our findings reveal a conserved role for wild-type Tau in restraining mitochondrial fusion and activity via mitofusins, highlighting its contribution to mitochondrial quality control and cellular stress resilience.

**OP9. Unravelling the molecular and functional maturation of the SST-expressing cortical interneurons during the first postnatal month.**

*Ourania Christodoulou[1,2], Konstantinos Diskos[1], Angeliki Velli[1,3], Panagiotis Moulos [2], Kyriaki Sidiropoulou[1,3], Myrto Denaxa[1,2,4]*

[1]Department of Biology, University of Crete, Heraklion, Greece

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Brain function is inextricably linked with the activity of cortical γ-aminobutyric acid-producing (GABAergic) interneurons (INs), which participate in the formation of inhibitory circuits and control the activity of excitatory glutamatergic pyramidal neurons (PNs). The main cortical INs (cINs), which contribute to around 60% of the total cIN population, include two cardinal IN types, defined by the expression of the calcium binding protein Parvalbumin (PV) and the neuropeptide Somatostatin (SST). The timeframe and mechanisms that underlie the maturation process of PV- and SST-expressing cINs, and their subtypes, remain mostly elusive. In the current thesis, we performed bulk RNA-seq, at critical developmental stages, to identify how the transcriptomic landscape of SST+cINs evolves during the first postnatal month. Our findings indicate that SST+ cINs undergo substantial molecular changes throughout the first month after birth, until they acquire their final mature characteristics and physiological properties. The above results were also in agreement with significant changes in the electrophysiological properties, both intrinsic and synaptic, of SST+ cINs, that were determined at the same developmental period. In summary, our results show that the maturation of SST-expressing cINs, during the critical period of the first month after birth, is a dynamic and plastic process. In addition, our work provides significant information on the mechanistic understanding of this process, which can be used to produce SST+ cINS, apt for stem cell therapies, and for elucidating the pathogenesis of interneuropathies.

**OP10. Investigating the neurotoxic effects of A30G a-synuclein mutation in C. elegans.**

*Christina Ploumi, Myrsini Kteniadaki, Leonidas Stefanis and Konstantinos Palikaras*

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The aggregation of α-synuclein is a hallmark of Parkinson's disease (PD) and various related neurological disorders. Several mutations in α-synuclein, including A53T, A30P, and the newly identified A30G, are associated with familial forms of PD. Our study aims to investigate the pathogenic mechanisms of the A30G mutation, recently identified in a Greek cohort of PD patients, to understand its impact on neurodegeneration, protein aggregation, and mitochondrial function. We generated transgenic Caenorhabditis elegans models expressing either A30G mutant or wild-type (WT) α-synuclein in dopaminergic (DA) neurons to evaluate neurodegenerative effects. Additionally, GFP-fused WT and A30G α-synuclein were expressed in the nematodes' body-wall muscle cells to assess aggregation. Mitochondrial membrane potential and reactive oxygen species (ROS) generation were measured in both WT and A30G-expressing nematodes to explore mitochondrial integrity. Our results indicate that C. elegans expressing A30G mutant α-synuclein in DA neurons exhibit accelerated DA neurodegeneration compared to WT α-synuclein-expressing animals, suggesting enhanced neurotoxicity. Aggregation assays in body wall muscle cells showed comparable aggregation phenotypes for A30G and WT α-synuclein, indicating that the mutation does not significantly alter aggregation propensity. However, A30G-expressing nematodes displayed increased mitochondrial membrane potential and elevated mitochondrial ROS generation, suggesting that A30G interferes with mitochondrial function, potentially leading to oxidative stress and cellular damage. These findings suggest that the A30G mutation in α-synuclein increases DA neuron vulnerability in C. elegans and disrupts mitochondrial homeostasis, evidenced by elevated ROS and membrane potential, without significantly altering a-synuclein aggregation. This model highlights the potential of A30G C. elegans PD models for fundamental research and drug screening. Such models may provide valuable insights into the molecular characterization of A30G pathogenicity and support the identification of targeted treatments for PD.

**OP11. When stress remodels the hippocampus: neuronal plasticity and behavioural implications**

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Anxiety disorders represent the most prevalent form of mental health disorders worldwide and they are associated with alterations in brain function. The hippocampus, a pivotal brain region involved in spatial memory and cognitive processing, is susceptible to anxiety-related states thereby impacting the synaptic plasticity of dendritic spines. Despite the significant research conducted on the impact of external stress, the role of genetically determined anxiety predisposition in hippocampal plasticity remains unclear. In the present study, we investigated the effects of endogenous anxiety predisposition on dendritic spine density in distinct hippocampal regions and examined the relationship between histological findings and spatial memory. To address this, adult female mice with High Anxiety–Related Behavior (HAB) and Normal Anxiety–Related Behavior (NAB) were utilized. These lines were selectively inbred for 40 generations based on anxiety-related behavior in the elevated plus maze. Dendritic spine density was analyzed across hippocampal subregions (CA1, CA3, and dentate gyrus, DG) using Golgi–Cox staining and quantitative image analysis. The results demonstrated that HAB mice exhibited a significant reduction in dendritic spine density in the CA1 region. Furthermore, a significant decrease in dendritic spine length was detected in the proximal stratum radiatum of the CA3 region. In the DG, no overall differences were observed, except for a spine density reduction in the exposed distal segment of neurons. Concurrently, the short-term spatial memory was assessed using the Y-maze test. HAB mice displayed comparable time spent and number of entries in the novel arm when compared to NAB mice, however they exhibited increased entries and longer time spent in the familiar arm of the Y-maze. These findings suggest alterations in spatial exploration rather than clear deficits in spatial memory. Overall, the results indicate that genetically determined anxiety predisposition is associated with specific, region-dependent alterations in hippocampal synaptic structure, accompanied by impairments in spatial exploration.

**OP12. Effects of smoking on renal function in the context of prediabetes and obesity in Drosophila Melanogaster.**

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Epidemiological studies in humans indicate that smoking increases renal failure risk significantly, while prediabetes and obesity are common causes of chronic kidney disease worldwide. The Malpighian tubules of Drosophila melanogaster exhibit functional similarities to human nephrons, and this their renal system could serve as a reliable model for renal diseases.

The aim was to investigate whether whole body cigarette smoke exposure in D. melanogaster causes renal system damage as evidenced by proteinuria and whether High Sugar Diet (HSD) or High Fat Diet (HFD) aggravates it.

Adult flies were fed with Normal Diet (ND), High Sugar Diet (HSD) or High Fat Diet (HFD)for 21 days. Flies were exposed to cigarette smoke (CS) via inhalation for 21 days, 20minutes per day. The glucose levels in hemolymph were measured colorimetrically. The body Mass Index (BMI) of flies was measured as body mass/total body length2. The survival of flies was estimated. Metabolic rate was estimated by CO2 production. Frass from was collected, and total protein was quantified, while uric acid levels were measured colorimetrically.

Flies fed with HSD and HFD had significantly higher glucose levels in the hemolymph of both sexes and their BMI was higher. Either with or without CS exposure, HSD and HFD increased the metabolic rate and significantly decreased the survival of the flies. The total protein/uric acid ratio was higher in male flies exposed to CS and in flies fed with HSD and HFD. Chronic exposure to CS, HSD and HFD affects the survival and metabolic rate and induces proteinuria in D. melanogaster.

Future studies will examine the gene expression of slit diaphragm components as well as the molecular mechanisms by which smoking, in combination with a high-sugar and high-fat diet, induces damage in nephrocytes.

**OP13. PPARδ Activation Balances Metabolic Impairment, Leading to Improved Cardiac Function in a Model of Experimental Diabetes.**

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Cardiovascular complications and metabolic disorders are closely interlinked, as the heart strongly depends on substrate availability for proper function. In type 1 diabetes (T1D), impaired insulin signaling increases cardiac fatty acid (FA) oxidation, leading to oxidative stress, inflammation, cell death, and ultimately diabetic cardiomyopathy (DCM). Here, we investigated whether pharmacological activation of PPARδ, a master regulator of lipid and glucose metabolism, could ameliorate the adverse metabolic effects and cardiac dysfunction associated with DCM. Experimental T1D was induced in male Wistar rats by a single streptozotocin (65 mg/kg) injection, followed by daily treatment with the PPARδ-specific agonist GW0742 (1 mg/kg) for 7 days. PPARδ activation attenuated the diabetes-induced left ventricular systolic dysfunction, as evidenced by increased fractional shortening and ejection fraction, and cardiomyocyte hypertrophy, another hallmark of DCM, as assessed by WGA staining of cardiac cryosections, compared with vehicle-treated diabetic rats. In addition, the elevated plasma levels of triglycerides and cholesterol were significantly lowered, and the rate of glucose utilization indicated an improving tendency, as assessed by intraperitoneal glucose tolerance test, after PPARδ activation in the diabetic group. Mechanistically, PPARδ activation upregulated the glucose transporters Glut1 and Glut4 and enhanced GLUT4 translocation to the plasma membrane, thereby promoting a metabolic shift towards glucose utilization in the diabetic myocardium. Transcriptomic analysis by RNA sequencing demonstrated enhanced metabolic flexibility and stress adaptation in diabetic versus treated rat hearts, with upregulation of fatty acid and branched-chain amino acid metabolism, PPAR/AMPK signaling and redox pathways. Concomitantly, extracellular matrix remodeling, hypertrophic growth as well as adrenergic and calcium signaling pathways were suppressed, consistent with reduced fibrosis and improved myocardial efficiency. These findings highlight PPARδ activation as a promising strategy to counteract cardiac and metabolic dysfunction in DCM.

**OP14. Investigation of the molecular mechanisms induced by lipopolysaccharide-inflammatory responses to a brain organoid model and their association with Alzheimer's disease.**

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Alzheimer Hellas (Greek Association of Alzheimer's Disease and Related Disorders)

Brain organoids represent an attractive three-dimensional system that enables the in vitro study of important physiological and pathological processes in the brain, thus constituting a promising alternative to animal models. The aim of the present study was to further investigate the potential of the novel 3D model that we recently developed to serve as an experimental system for the study of brain pathophysiology. For this purpose, we generated brain organoids from the tissue of newborn Wistar rats. Brain cells, isolated by enzymatic digestion, were cultured in the presence of a scaffold (BME) in an appropriate growth medium for 28 days. Subsequently, the developed organoids were exposed to lipopolysaccharides (LPS), which were replenished in the growth medium every 48 hours from day 28 to day 42. On day 42, the organoids were either lysed for protein extraction or fixed and embedded in paraffin. Immunohistochemical analysis confirmed the presence of neurons, astrocytes, and microglial cells by day 28. Moreover, LPS exposure increased the production of Aβ and phosphorylated tau (p-tau181), as demonstrated both by immunohistochemistry and Western blot analysis. The increase in Aβ was associated with a parallel increase in the amyloid precursor protein (APP), the levels of which doubled as a result of chronic exposure to LPS. This was accompanied by an early inflammatory response, as demonstrated by the elevated levels of IL1β. In addition, microglial activation (IBA1) and the presence of apoptotic markers (caspase 3) were demonstrated by immunohistochemistry. Overall, these findings suggest that the brain organoids developed from newborn rat braincells responded to inflammatory stimulation and can develop an Alzheimer's disease–like pathology. This model provides a reliable platform for studying inflammatory mechanisms and molecular pathophysiological pathways in the brain. Moreover, it is a promising system for the evaluation of potential therapeutic interventions in vitro.

**OP15. Maternal IGHG locus duplications impair infants' passive immunity.**

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Infants depend on passive immunity to safeguard them against infections during the first months of life. Maternal immunoglobulin G (IgG) antibodies are actively transported across the placenta and confer this protection. In this study, we discovered common, but previously unrecognized, naturally occurring gene fusions between loci encoding IgG1and IgG4 subclasses that impair the transplacental IgG transport. These gene fusions result from gene duplications combining regulatory elements of the Immunoglobulin Heavy Constant Gamma (IGHG1) gene with IGHG4-like constant regions. Mothers with these duplications generate antibodies that are less efficiently transferred to the fetus, resulting in lower antibody levels in newborns and a higher risk of respiratory infections during infancy. Our insights warrant consideration in the development of personalized vaccination strategies during pregnancy to better protect infants against infectious diseases.

# POSTER PRESENTATIONS

**Saturday March 14th, 2026**

**8:30 - 9:30 Foyer of the Auditorium Hippocrates**

**PP1-PP10 Chairs**

**Assoc. Prof. Isaac Aidonidis (UTH) & Assoc. Prof. Roxane Tenta (HU)**

**PP11-PP20 Chairs**

**Asst. Prof. Erasmia Rouka (UTH) & Asst. Prof. Marietta Armaka (AUTH)**

**PP21-PP30 Chairs**

**Assoc. Prof. Sophia Lavrentiadou (AUTH) & Asst. Prof. Georgios Markopoulos (UoI)**

**PP1. Effect of mechanical loading and/or treatment with natural/synthetic compounds on human cells with osteoblastic phenotype**

*Vasiliki Vitsou[1], Kyriaki Kyriakidou[2], Eirini Chatzinikita[3], Ioannis Kostakis[4], RoxaneTenta[1], Anastassios Philippou[3]*

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Bone remodeling functions as an ongoing process throughout life to preserve skeletal strength. Bone remodeling processes are particularly influenced by mechanical loading. Bone tissue cells possess the ability to detect mechanical stimuli including tension and compression forces. Therefore, bone cells initiate signaling pathways which interfere with gene expression and cell differentiation processes. As recent scientific interest focuses on the effect of natural and synthetic antioxidant molecules on osteoblast physiology, the aim of the present study was to investigate a)how MG-63 osteoblast-like cells respond to mechanical stimulation and b) how the natural polyphenol hydroxytyrosol (HT) and the HT synthetic analog (FR503) impact this cellular response. MG-63 cells were plated in special collagen-coated plates, were treated with HT andFR503 and were then exposed to cyclic tensile strain for 12 hours using a cell-stretching apparatus (Flexcell Tension System). Fluorescence-activated cell sorting (FACS) was used to analyze cell cycle distribution and apoptosis in both treated and control MG-63cells. Finally, the alteration of the expression of five selected bone-related genes were evaluated by Real-Time PCR. Our results revealed the protective role of mechanical loading in the MG-63 osteoblast-like cells distribution and in reducing apoptosis in all tested conditions. Additionally, enhanced gene expression of osteocalcin (OSC), bone-specific alkaline phosphatase (ALP) and bone morphogenetic protein 7(BMP7) were observed after combining mechanical loading and HT and FR503 treatment conditions. Our findings suggest that more profound studies of these loading-mediated cellular responses might help the development of new approaches to enhance bone re-generation and tackle bone-related diseases.

**PP2. Targeting SRPK1/2 Kinases for Neuroprotection under Oxidative Stress**

*Maria-Panagiota Sarafi[1], Andreas P. Katsenos[1,2], Efstathia Kotoula[1], Stela Duro[1], Dioni Zoidi[1], Argiris Katsakoulas[1], Aliki Kolokytha[1], Foivos S Kanellos[1], Rea Gklinavou[1], Panagiota Tzitiridou[1], Yannis V. Simos[1,2], Lampros Lakkas[1,2], GeorgiosMarkopoulos[1,2], Dimitrios Peschos[1,2], Konstantinos I. Tsamis[1,2,3]*

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-β accumulation and the microtubule-associated protein tau hyperphosphorylation. A mounting body of evidence suggests that dysregulation of alternative splicing plays a substantial role in AD pathobiology, particularly through altered splicing of the MAPT gene, which encodes tau. The serine/arginine-rich protein kinasesSRPK1 and SRPK2 regulate alternative splicing by phosphorylating SR splicing factors and modulating exon selection, including MAPT exon 10, which determines the balance between 3R and 4R tau isoforms. Excessive production of 4R tau has been associated with tau aggregation and neuronal dysfunction.

Following the experimental identification of SRPK expression in the brain and extensive literature analysis of their selective inhibitor SRPIN340, its effects were investigated invitro using primary hippocampal neuron cultures derived from early postnatal rats. Neurons were maintained under physiological conditions or subjected to oxidative stress induced by hydrogen peroxide ($H_2O_2$, 100 μM), a well-established model that mimics initial stages of neurodegenerative disorders including AD. The primary objective was to investigate whether SRPK1/2 inhibition could attenuate oxidative stress–induced neurodegeneration without eliciting toxicity. Cell viability, cytotoxicity and morphology were assessed through standard assays and quantitative analysis of dendritic length, dendritic spine density, and dendritic field complexity. Pharmacological inhibition of SRPK1/2 significantly improved neuronal viability and reduced cytotoxicity in $H_2O_2$-exposed neurons. Moreover, SRPIN340 preserved dendritic architecture, enhanced spine density, and improved dendritic field expansion in neurons, supporting a neuroprotective role for SRPK inhibition and highlighting SRPK-dependent pathways as potential therapeutic targets in AD

**PP3. HIF-1α association with cohesin complex drives triacylglycerol biosynthesis under hypoxia**

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Adaptation to low oxygen, termed hypoxia, is fundamentally important in various diseases, including cancer. Hypoxia causes metabolic and transcriptional reprogramming, which is enabled by a small family of transcriptional factors called HIFs (Hypoxia Inducible Factors). HIFs exist in three main isoforms and consist of one alpha and one beta subunit. The oxygen regulated HIF-1α isoform is heavily modified by many post-translational modifications, which affect its stability, subcellular localization and interactions with other proteins (1). Analysis of mass spectrometry results of proteins co-immunoprecipitated with HIF-1α, suggests that HIF-1α potentially interacts with theSMC1A subunit of the cohesin complex, which marks the boundaries of topologically associated domains (TADs) on chromatin (2). The association between HIF-1α andSMC1A was verified by HIF-1α immunoprecipitation and western blotting. To map theHIF-1α domain that mediates their association, SMC1A cDNA was introduced into PGEX-4T-1 bacterial expression vector. GST-SMC1A was overexpressed in bacteria, purified and subjected to in vitro pull-down assays with purified HIF-1α fragments. Our results have shown the direct binding of the HIF-1α C-terminal domain with SMC1A. Moreover, CLIP (Cross-linking and immunoprecipitation) experiments from extracts of cells stably expressing different phospho-deficient or phospho-mimetic HIF-1α forms showed thatHIF-1α phosphorylation by ERK1/2 enhances the HIF-1α/SMC1A interaction, something that was also confirmed by immunofluorescence microscopy. Finally, by combining proteomic and transcriptional analysis with results of phenotypic experiments, we discovered that HIF-1α/SMC1A association could greatly enhance triacylglycerol biosynthesis in hypoxic cancer cells. Taken together, our results have revealed a novelHIF-1α interaction with SMC1A, which facilitates the reprogramming of lipid metabolism under hypoxia. This is potentially mediated by cohesin-dependent chromatin remodeling, a hypothesis which is currently under investigation.

References: 1. Yfantis et al., Cells, 2023; 2. Rittenhouse et al., Current Opinion in Genetics & Development, 2024

**PP4. Exosome-derived proteins as candidate biomarkers in progression of Parkinson's diseaseand its correlation with clinical parameters.**

*Stela Duro[1], Foivos S. Kanellos[1,5], Martina Samiotaki[4], Theologos Michailidis[3], Spyridon Konitsiotis[2], Lampros Lakkas[1,5], Ioannis Simos[1,5] ,Georgios Markopoulos[1,5], Dimitrios Peschos[1,5], Konstandinos I. Tsamis[1,2,5]*

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Objective: In this study extracellular vesicle (EV) serum proteins were isolated and characterized to explore their potential as reliable biomarkers for the progression of Parkinson's Disease(PD). Concurrently, the clinical profile of patients was evaluated to finally investigate its association with the exosome-derived protein levels.

Background: Parkinson's disease is the second most prevalent neurodegenerative disorder, posing substantial challenges in both accurate diagnosis and effective management. It is a complex disease, influenced by multiple factors that contribute to overall clinical manifestation of the disease. Simultaneously, exosomes have emerged as a promising source of biomarkers, as they enclose a range of proteins implicated in the pathophysiology of the disease [1].

Methods: A group of 36 patients with PD was followed up for one year. Blood serum samples were collected at two timepoints (baseline and follow-up). EVs were isolated through sequential centrifugation, and their protein content was characterized using mass spectrometry. Clinical evaluation of symptoms was conducted at these timepoints using the MDS-UPDRS scale.

Results: Out of a set of 291 proteins analysed, we identified 94 with statistically significant differences in abundance between baseline and follow-up samples. Three of these proteins showed strong correlations with clinical progression markers, including age, motor symptoms, and the bradykinetic/axial phenotype. These proteins are involved in processes related to inflammaging, immune regulation, and vascular remodeling.

Conclusions: The findings suggest that proteins identified in extracellular vesicles represent a promising step toward the discovery of biomarkers for disease progression. Furthermore, our results

support the hypothesis that disease progression is governed by a dynamic equilibrium between chronic neuroinflammatory burden and compensatory neuroprotective mechanisms.

References:[1] Pinnell JR, Cui M, Tieu K. Exosomes in Parkinson disease. J Neurochem. 2021May;157(3):413-428. doi: 10.1111/jnc.15288.

**PP5. Colistin effects on heart tissues in a "sepsis-like" animal model.**

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Sepsis is a life-threatening condition involving immunological dysregulation during infection mainly from Gram-negative bacteria having the polysaccharide LPS on their surface. In Intensive Care Units colistin is used to treat mainly multidrug resistant microbes. Even though effective, colistin has been reported to induce muscular atrophy and toxic effects. The aim of the study was to evaluate effects of colistin in hearts from adult male Wistar rats, using a "sepsis-like" model. A single dose of 2.5mg/kg LPS was administered intraperitoneally, followed -5 days later- by a daily dose of colistin(150000U/kg) alone or combined with vasoconstrictors for 10 days. Animals' weight was monitored daily and heart tissues were harvested after euthanasia. In-silico network pharmacology analyses were performed to evaluate the drug interactions with cellular signaling pathways. Heart weight and curvature were measured and tissues were used for colistin levels evaluation and Western blots. Furthermore, 5μm heart slices were used for fibrosis evaluation, using Picrosirius red staining. Animals' body weight declined 24hafter LPS treatment and maintained lower compared to control in both LPS-saline and LPS-colistin treated animals, indicating the "sepsis-like" phenotype and possible subclinical drug toxicity. Heart weight and area were also reduced, with heart curvature being altered in colistin groups, indicating early remodeling. Additionally, colistin levels were increased in LPS treated hearts, while vasoconstrictors appear to reduce this effect. Furthermore, no activation of apoptosis (cleaved caspase-3 and PARP) was observed while elevated levels of LC3 and beclin proteins were detected in colistin treated hearts, indicating autophagy activation. Finally, increased levels of collagen fibers were detected in colistin treated hearts demonstrating tissue fibrosis. To conclude, our data show that colistin appears to induce alterations in heart tissues especially in septic conditions, but the exact mechanism needs to be further evaluated.

**PP6. Estimation of the toxicity of cisplatin on zebrafish (Danio Rerio) embryos.**

*Leonardos Maximos[1,3], Karavida Vasileia[1,3], Leonardos Dimitrios[4], Simos V Yannis[1,2], Tsamis Konstantinos[1,2], Markopoulos Georgios[1,2], Peschos Dimitrios[1,2], Lakkas Lampros[1,2]*

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Cisplatin, also known as cisplatinum or cis-diamminedichloroplatinum (II), is a widely used chemotherapeutic agent. It has been employed in the treatment of a broad range of human malignancies, including bladder, head and neck, lung, ovarian, and testicular cancers. Cisplatin shows efficacy against multiple cancer types, such as carcinomas, germ cell tumors, lymphomas, and sarcomas. Its antitumor activity is primarily attributed to its ability to form crosslinks with purine bases in DNA, thereby disrupting DNA repair processes, inducing DNA damage, and ultimately triggering apoptosis in cancer cells. The aim of the present study is to determine the effect of cisplatin on zebrafish (Danio rerio) embryos. For this purpose, exposure of dichorionated zebrafish embryos 24 hours postfertilization (24hpf) to solutions of different concentrations of cisplatin was performed. The exposure lasted 96 hours. The experiments aim to determine the toxicity of cisplatin, but also the general effect of the substance. At the concentrations studied (10uM, 25uM,50uM, 100uM, 200uM and 400uM) no mortality of exposed embryos was observed. The overall effect of the exposure was studied by 5 different factors: body length, eye size, heart beats per minute, behavioral analysis and study of the cell cycle. The results indicate that body length, eye size and heart rate were not affected from the exposure. On the other hand, the behavior of exposed embryos was affected, especially at the highest dose (400uM). Also the cell cycle of the exposed embryo was affected, presenting a dose-dependent manner, beginning from the concentration of 50uM.

**PP7. Monitoring the effect of taxol on exposed zebrafish (Danio Rerio) embryos.**

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Taxol, also known as paclitaxel, is a chemotherapeutic agent widely used in the treatment of various cancers. Since the discovery of its antitumor activity, Taxol has been administered to more than one million patients, making it one of the most extensively used anticancer drugs. It was the first microtubule-targeting agent described in the literature, and its primary mechanism of action involves the disruption of microtubule dynamics, leading to mitotic arrest and subsequent cell death. In addition to this principal mechanism, several secondary apoptotic pathways have also been reported. Despite its widespread clinical application, Taxol presents several limitations, including the need for environmentally sustainable production methods, improved bioavailability without adverse effects, and the reduction of drug resistance observed in a significant proportion of treated cells. The aim of the present study was to investigate the effects of Taxol exposure on zebrafish embryos. Dichorionated zebrafish embryos at 24 hours post-fertilization (24 hpf) were exposed to different concentrations of Taxol for a period of 96 hours. The concentrations tested were 100 nM, 250 nM, 500 nM, 1000 nM, 2000 nM, and 4000 nM. No mortality was observed at any of the concentrations studied. The overall impact of Taxol exposure was assessed using five parameters: body length, eye size, heart rate, behavioral analysis, and cell cycle evaluation. The results demonstrated that body length and heart rate were not significantly affected by Taxol exposure. In contrast, eye size and behavioral responses were affected, particularly at the highest concentration (4000 nM). Analysis of the cell cycle revealed no significant changes in exposed embryos.

**PP8. Beyond Multiple Sclerosis: Exploring the Spectrum of Autoimmune-Mediated Demyelination.**

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) marked by neuroinflammation, myelin loss, and axonal damage. Its diagnosis remains challenging due to significant clinical and radiological overlap with other conditions, particularly systemic autoimmune diseases (SADs), which can also present with optic neuritis, transverse myelitis, and white matter lesions. The absence of disease-specific biomarkers can result in MS being diagnosed using the McDonald criteria, which rely on excluding alternative diagnoses. As a result, misdiagnosis is common, with nearly 20% of patients initially diagnosed with MS later found to have other disorders, many of them autoimmune, leading to inappropriate treatment and increased disability. Previous work by our group in a cohort of 193 patients presenting with CNS demyelination showed that while 66.3% were diagnosed with MS spectrum disorders,24.9% were classified as having CNS autoimmune disease. Of these, 6.7% fulfilled criteria for a defined SAD, while 18.1% were categorized as having demyelinating disease with autoimmune features (DAF), characterized by demyelination and autoimmune markers without meeting SAD criteria. The aim of this study was to characterize the peripheral blood transcriptomes of MS, SAD, and DAF patients using bulk RNA sequencing, to identify distinct molecular signatures. Treatment-naïve blood samples were collected at first demyelinating presentation, and patients were classified after multidisciplinary evaluation and one year of follow-up. Transcriptomic analysis revealed minimal overlap among groups, with only 22 commonly upregulated genes. MS patients showed enrichment of coagulation-related pathways, SAD patients demonstrated activation of innate immune responses, and DAF patients exhibited overexpression of genes related to cytoskeletal organization. These findings highlight distinct immunopathogenic mechanisms underlying similar clinical presentations and underscore the need for molecular biomarkers to improve diagnostic accuracy.

**PP9. Operational Safety in Long-Haul Drivers: The Influence of Cardiovascular Risk, Sleep and Functional Capacity: A Pragmatic Cross-Sectional Field Study.**

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Introduction: Professional long-haul drivers operate under demanding occupational conditions that substantially elevate cardiovascular risk. Despite this, simple markers of functional capacity remain largely overlooked in occupational health screening. Aims: The purpose of the current study is to thoroughly examine the multifaceted relationship between the physical and mental health of professional drivers and their involvement in traffic accidents, integrating evidence on prevailing health conditions and their direct impact on operational safety. Material and Methods: This study included 102 professional long-haul drivers (48.4 ± 10.7years) who underwent comprehensive anthropometric, cardiovascular, functional and biochemical assessments. Sleep quality, fatigue, and psychological status were evaluated using validated questionnaires and drivers were classified according to sleep quality and cardiovascular risk. Results: Poor sleepers exhibited significantly worse psychological status, quality of life, fatigue and sleep-related scores compared with good sleepers; however, sleep quality was not associated with differences in cardiometabolic parameters or cardiovascular risk($p > 0.005$). Drivers at high cardiovascular risk were significantly older and demonstrated lower handgrip strength. Age was independently associated with increased cardiovascular risk (OR= 1.42, $p < 0.001$), whereas handgrip strength showed a protective association(OR= 0.88, $p = 0.035$).Conclusions: Cardiovascular risk in professional drivers is driven primarily by age and functional capacity rather than subjective sleep complaints, positioning handgrip strength as a robust, low-cost screening tool for cardiovascular vulnerability in this high-risk occupational group.

**PP10. Non-Invasive Cardiovascular Assessment in Conscious Rodents: Physiological Insights and Limitations of the CODA Tail-Cuff System.**

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The CODA tail-cuff system (Kent Scientific®, USA) provides a non-invasive approach for longitudinal cardiovascular assessment in conscious rodents. Beyond standard blood pressure measurements, CODA-derived data capture adaptive, stress-related, and autonomic signatures that are highly sensitive to experimental handling, circadian timing, and environmental stimuli. Here, we outline the physiological information directly and indirectly derived from CODA recordings, identify major methodological limitations, and describe common experimental failure modes that influence data interpretation. Male Wistar rats were handled and standardized restrained for cardiovascular assessment from PD30 to PD59 either once per day or repeatedly within 24 hours (0, 2, 4, 6, 12 and24h). According to our results, no developmental cardiovascular changes were observed with Mean Arterial Pressure (MAP), Systolic and Diastolic blood pressure varying from105.7±4.75, 127.5±2.65, 95.4±5.77, n=4 at PD35 to 109.8±3.02, 127.2±3.50, 101.6±2.95, n=4 at PD59, respectively. Individual animals demonstrated remarkable baseline stability, confirming that acclimatization and handling practices normalize cardiovascular responses independent of developmental age. Critical differences emerged between measurement protocols. Resilient animals recovered completely within 24 hours after repeated same-day measurements, demonstrating intact autonomic buffering and baroreflex capacity. Conversely, daily measurements in stress-sensitive animals produced progressive dysregulation over 7 consecutive days (e.g. Rat3 PD38 MAP=101.4± 8.6,PD44 MAP=125.3± 14.4, $p<0.05$), indicating the need of 2-3 days resting period between measurements. CODA recordings are affected by restraint-induced sympathetic activation, novelty stress, and environmental stimuli including temperature-dependent tail vasomotor tone and olfactory/auditory startle responses. Circadian rhythm-dependent baseline shift and researcher-dependent variables (handling style, experimenter sex, hormonal status) introduced additional variability. Overall, PD30-PD59 animals exhibit cardiovascular stability suitable for pharmacological studies when proper habituation is applied. Avoiding daily or same-day CODA measurements is essential to minimize stress effects,

improve data reliability, and distinguish true treatment effects from handling- or age-related adaptation.

**PP11. Intraoperative flow cytometry for adrenal gland malignancies: proof of concept.**

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Background Intraoperative assessment of adrenal gland malignancies remains challenging and relies primarily on postoperative histopathological evaluation, which represents the diagnostic gold standard. In recent years, intraoperative flow cytometry has emerged as a rapid and quantitative tool in the evaluation of several malignancies, including brain, breast, colorectal, and hepatic tumors, where it has been used to assess proliferative activity, ploidy and tumor margin status in real time. However, its application in adrenal gland malignancies has not been systematically explored.

Methods Fresh adrenal gland tissue samples were collected intraoperatively from three patients undergoing adrenalctomy for suspected malignancy. Samples were immediately processed to generate single-cell suspensions and analyzed by flow cytometry. DNA content and cell cycle distribution were assessed using propidium iodide staining. Final histopathological evaluation served as the reference (gold standard) for comparison.

Results Intraoperative flow cytometry was technically feasible in all cases, enabling rapid acquisition of quantitative cellular data. Distinct DNA content and cell cycle profiles were identified among samples. Flow cytometric findings were interpretable in the context of the corresponding histopathological diagnoses, supporting the biological relevance of the observed patterns.

Conclusions This proof-of-concept ongoing study demonstrates the feasibility of intraoperative flowcytometry in adrenal gland malignancies. Building on its established utility in other solid tumors, intraoperative flow cytometry may offer rapid, complementary information alongside histopathology during adrenal surgery. Analysis of a larger patient number is required to determine its diagnostic accuracy and clinical value.

**PP12. Optimizing Laboratory-Familiar Histochemical Stains for Sperm DNA Fragmentation Assessment in a Modified Sperm Chromatin Dispersion Protocol.**

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Genome integrity of bovine spermatozoa is widely recognized as a critical factor in evaluating fertility and as an essential determinant for blastocyst formation. Sperm DNA fragmentation is an important biomarker of fertility and has been shown to correlate with key physiological parameters of sperm. The Sperm Chromatin Dispersion (SCD) test provides a simple, practical and cost-effective tool for assessing DNA fragmentation; however, the diagnostic reliability depends heavily on the staining technique used to visualize halos of dispersed chromatin. This study evaluated and optimized five commonly used histochemical stains for application in a modified SCD protocol, to enhance accuracy and ease of interpretation. Frozen–thawed semen samples from five fertile bulls were washed to remove cryoprotectants, embedded in agarose on precoated slides, and subjected to acid denaturation and lysis to generate chromatin halos. Slides were then stained with Toluidine Blue (TB), Giemsa (G), May Grünwald–Giemsa (MGG), Feulgen & Rossenbeck (F&R) and Haematoxylin & Eosin (H&E). TB and G failed to provide consistent or distinguishable halos. MGG modified protocol enabled halo visualization but introduced notable non-specific background staining that reduced distinctiveness of halos. Specific modified protocols of F&R and H&E also visualized halos but reacted variably with agarose: F&R did not produce non-specific background staining while H&E did. This study highlights that optimized histochemical staining is crucial for accurate SCD-based DNA fragmentation assessment in bovine spermatozoa. Modified MGG, H&E, and F&R produced reliable halos, with MGG and H&E recommended for routine labs. Further studies should be conducted to implement the above experiments to other animal species and/or breeds.

**PP13. Exaggerated cardiovascular drift in iron-deficient individuals during exercise in the heat.**

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Introduction: Anemia is characterized by altered cardiovascular function during exercise, due to decreased oxygen delivery. As global temperatures continue to rise due to climate change, individuals exercising in the hot environments experience additional thermal and cardiovascular strain that further challenges cardiovascular stability. Thus, we aimed to explore the combination of iron deficiency anemia and heat stress on cardiovascular responses during submaximal exercise. Methods: Nine anemic (5 F, 4 M) and nine control(5 F, 4 M) individuals, matched for anthropometric characteristics but different by design in hematological parameters and physical fitness, participated in the study. They cycled in heat 33 °C with 40% relative humidity (RH) at an intensity ~10% below the ventilatoryt hreshold until achievement of 1 °C increase in rectal temperature. Hemodynamic parameters were continuously recorded throughout exercise via finger plethysmography (Finometer). Results: Anemic participants exhibited a more pronounced cardiovascular drift. Specifically, the increase in heart rate (HR) and the decrease in stroke volume (SV)were significantly greater in anemic compared to non-anemic individuals (p0.05).Moreover, mean arterial pressure (MAP) was significantly lower in the anemic group throughout the entire exercise period (p=0.04). Conclusion: Anemic individuals showed increased cardiovascular strain, as depicted from HR and SV responses, compared to controls during submaximal cycling in the heat. This finding has meaningful implications given the high prevalence of anemia in various cardiovascular conditions, as well as the elevated rates of iron deficiency anemia in athletic population who frequently train in hot environments.

**PP14. From miR-34c to cryopreservation-induced oxidative stress: A comparative computational analysis of genes and pathways in sperm.**

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MicroRNAs (miRNAs) are small, regulatory, non-coding RNA molecules, 18–22nucleotides in length, which bind to target genes and regulate gene expression at the post-transcriptional level, primarily through translational repression (1). In the context of male reproduction, miRNAs have been studied for their involvement in sperm production, differentiation and function. A prominent example is the evolutionarily conserved miR-34c.Although most studies have focused on the tumor-suppressive role of miR-34c, its broader physiological functions remain largely underexplored (2). Recent data have associated miR-34c with spermatogenesis and subfertility through its role in the regulation of apoptosis (3–7). Moreover, miR-34c is closely related to pathways implicated in oxidative stress, such as those activated upon sperm cryopreservation. To investigate the potential regulatory role of miR-34c under conditions of oxidative stress, a computational workflow was developed integrating two target prediction tools (TargetScan, DIANAmicroT-CDS) and two experimentally validated databases (miRTarBase, TarBase), aimingto identify overlapping genes between miR-34c targets and Gene Ontology–annotated genes related to oxidative stress. The resulting gene set served as the basis for the construction of protein–protein interaction (PPI) networks and enrichment analyses, performed independently for Homo sapiens and Bos taurus. Comparative cross-species analysis highlighted candidate genes with well-documented involvement in cellular stress-response pathways. Overall, this approach provides a structured framework for miRNA-based gene prioritization and supports ongoing RT-qPCR experiments aimed at linkingmiR-34c expression with functionally relevant target genes in bull sperm under oxidative stress conditions.

**PP15. Selective AT2R agonism mitigates H2O2-induced dendritic retraction and spine depletion in primary hippocampal neurons.**

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The Renin-Angiotensin System (RAS) is increasingly recognized as a critical regulator of neurodegenerative processes, extending beyond its traditional role in systemic physiology. While the classical RAS axis, mediated by the Angiotensin II Type 1 Receptor (AT1R), promotes oxidative stress and neuroinflammation, the alternative axis acting through the Angiotensin II Type 2 Receptor (AT2R) exerts vital neuroprotective counter-responses. This study investigates the neuroprotective potential of Y6AII, a novel synthetic AT2R agonist engineered for high receptor specificity via a tyrosine substitution at the sixth amino acid position. Using an in vitro oxidative stress model, primary hippocampal pyramidal neurons were isolated from newborn wild-type rats and maintained in a 14-day culture. Neurotoxicity was induced via a validated concentration of hydrogen peroxide H2O2 (100 μM), administered as a co-treatment with Y6-AII across a concentration range of 0.5 to 10 μM. Quantitative morphometry revealed that Y6-AIIsignificantly mitigated the deleterious structural effects of H2O2. Notably, co-treatment with 1 μM of the agonist proved to be the most effective concentration, successfully preventing dendritic retraction and restoring spine density to levels statistically in distinguishable from control cultures. Sholl analysis further confirmed the preservation of dendritic arborization complexity (one-way ANOVA, $p < 0.05$), without compromising overall cell viability. These findings demonstrate that selective AT2R activation via Y6-AIIconfers a robust neuroprotective profile, acting as a prophylactic shield against oxidative degradation. By preventing the progressive loss of neuronal structural plasticity, this study identifies Y6-AII as a dynamic target for preventive therapeutic interventions aimed at halting the early stages of neurodegeneration before irreversible structural damage occurs.

**PP16. Cellular and Cognitive Effects of Ketamine in Chronic Alcohol Exposure.**

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Alcohol Use Disorder (AUD) constitutes a chronic, relapsing brain disorder associated with significant cognitive impairment and limited therapeutic options. Chronic alcohol exposure disrupts hippocampal function, synaptic plasticity, and neurogenesis, leading to deficits in learning and memory. Within this context, ketamine has emerged as a promising pharmacological candidate due to its rapid neuromodulatory and neuroplastic effects; however, the underlying neurobiological mechanisms remain insufficiently understood. The present study aimed to investigate the effects of a single sub-anesthetic dose of ketamine on behavioral, cellular, and neuroplastic alterations induced by chronic alcohol consumption, with particular emphasis on the hippocampal dentate gyrus. Using preclinical rat models of induced alcoholism, spatial memory and behavior were assessed through established behavioral paradigms. In parallel, cellular and molecular analyses were performed to examine glial cell distribution and activation, granule cell morphology, synaptic plasticity markers, and potential adaptations in hippocampal neurogenesis. Our findings demonstrate that chronic alcohol exposure significantly impairs spatial memory and behavioral performance, accompanied by pronounced alterations in hippocampal cellular organization and plasticity-related processes. Acute ketamine administration partially reversed alcohol-induced behavioral deficits and was associated with distinct neurobiological changes within the dentate gyrus, including modulation of glial activity and granule cell plasticity, as well as indications of enhanced neurogenic responses. Overall, these results provide novel insight into the multilevel effects of ketamine on hippocampal physiology under conditions of chronic alcohol exposure. By linking behavioral outcomes with cellular and neuroplastic mechanisms, the study supports the potential therapeutic relevance of ketamine in mitigating cognitive and neurobiological deficits associated with Alcohol Use Disorder and underscores the need for further investigation into its mechanism of action.

**PP17. Investigating the role of the 'Hes3 Signaling Axis' in MASH-related hepatocellular carcinoma.**

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by abnormal hepatic fat accumulation and represents a global epidemic closely associated with obesity. MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH) with hepatic fibrosis, markedly increasing the risk of hepatocellular carcinoma (MASH-HCC), a malignancy with limited therapeutic options. Notch signaling is a critical pathway in HCC-related processes and includes a recently described, less-studied non-canonical branch termed the 'Hes3 Signaling Axis'. Hes3 signaling can be co-opted by both normal and cancer cells as an alternative growth and survival mechanism during injury, regeneration, and oncogenesis, playing a notable role in stem cell dynamics. Aim: This study investigates the Hes3 Signaling Axis as a potential alternative survival mechanism contributing to oncogenesis within the MASH-HCC liver microenvironment. Methods: MASH-HCC development was modeled in vivo using two animal models: (a)adult male C57BL/6J mice subjected to a Western diet combined with carbon tetrachloride (WD/$CCl_4$) to recapitulate different stages of human MASH-HCC at defined time points, and (b) the STAM model, involving a single postnatal streptozotocin injection followed by ad libitum high-fat diet feeding. Hes3 expression was assessed in tumoral and peri-tumoral liver tissues. In vitro, HepG2 cells were cultured under serum-rich (Serum+) or serum-free/JAK-inhibiting (Serum−) conditions known to induce Hes3 signaling. Hes3 expression and sensitivity to Hes3-siRNA or Hes3-targeted compounds were evaluated using cell counting and metabolic assays. Results: Hes3 expression was significantly elevated in peri-tumoral tissues compared to tumors in MASH-HCC mice, while steatosis or fibrosis alone did not induce Hes3 upregulation. Serum− HepG2 cells showed increased Hes3 expression and greater sensitivity to Hes3-targeted interventions. Conclusion: These findings suggest that Hes3 expression emerges during late-stage MASH-HCC and might exhibit marked heterogeneity in the tumoral and/or peritumoral MASH-HCC microenvironment, warranting further investigation of a potential $Hes3^+$ cellular subtype that requires diversified therapeutic strategies.

**PP18. CPR performance in school children: possible modulating factors and skill retention.**

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Introduction: Early CPR training at around 12 years of age is promoted as a public health strategy to improve bystander response and survival, yet evidence on short-term skill retention and the influence of anthropometric characteristics in children remains limited. Aims: The aim of the present study was to assess CPR skill retention in children after a 3-hour structured training session and to explore the possible influence of anthropometric characteristics on CPR performance over time. Material and Methods: Following appropriate ethics consent procedures (Ethics Committee of the University of Thessaly; Approval No. 4-1/8-2/2023). Seventy-one children (34M/37F; 12.0 ± 0.3 years) participated in a 3-hour structured CPR training program focused on chest compressions. CPR quality was assessed one week and one-month later using quantitative metrics (Little Anne QCPR®, Laerdal Medical Inc.,Stavanger, Norway), paired with a mobile application to provide real-time measurement. High-quality CPR was defined as >70% correct performance in rate (100-120/min), depth(50-60 mm), and complete release, recorded over one minute per student. Anthropometric characteristics were recorded and their association with CPR performance was examined. Results: By only undertaking a 3-hour training once, thirty-seven children achieved the predefined success criterion of >70% correct compressions, corresponding to a success rate of 53%. The success score remained largely unchanged also at one week and one month after training (p= 0.803). CPR quality was not influenced by body weight, body height, BMI, (p> 0.05). These results indicate, a good success rate and excellent skill retention over time, independently from children's anthropometric characteristics. Conclusions: 3-hour CPR training session results in chest compression skill development and skill retention in children aged 12 years old, regardless of body weight or BMI. These findings support the feasibility and effectiveness of implementing CPR training programs in pediatric populations, reinforcing their value within school-based and community based educational initiatives.

**PP19. Characterization of the cardiac endothelial cell proteome during progression of heart failure with preserved ejection fraction in the background of obesity and diabetes mellitus.**

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Introduction: Cardiovascular disease constitutes a leading cause of mortality worldwide, with heart failure with preserved ejection fraction (HFpEF) being the predominant form in patients with diabetes and obesity. Although the contribution of cardiac endothelial cells to the oxidative and pro-inflammatory tissue microenvironment is recognized, the precise molecular mechanisms linking endothelial dysfunction to the clinical presentation of the disease remains unclear.

Methods: Male C57BL6 mice (8 weeks old) were divided into two groups: a) low-fat diet (Normaldiet, ND) and b) high-fat diet (HFD) combined with a nitric oxide synthase inhibitor (L-NAME) in water (HFpEF diet) for 5, 10, or 15 weeks. Phenotypic and biochemical assessment of pre-diabetic characteristics, echocardiographic and histological validation of the HFpEF phenotype, and proteomic analysis on isolated cardiac endothelial cells were performed. In parallel, plasma proteomic analysis was conducted on four human groups: healthy controls, patients with HFpEF, patients with Type 2Diabetes (T2D) without heart failure, and patients with T2D and HFpEF.

Results: HFpEF mice exhibited an obese phenotype and elevated blood glucose levels consistent with diabetes development. Significant increases in systolic and diastolic blood pressure, along with changes in key echocardiographic parameters, were observed in the HFpEF group. Histological analyses revealed myocardial hypertrophy and fibrosis in HFpEF mice. Proteomic analyses of isolated endothelial cells indicated significant molecular factors potentially participating in early cardiac endothelial remodeling, favoring HFpEF-associated endothelial dysfunction. Comparative analysis inhuman samples investigated whether observed tissue alterations are reflected in the plasma proteomic profile of diabetic patients developing HFpEF.

Conclusions: Identifying biological pathways differentiated in cardiac endothelial cells during HFpEF may contribute to the discovery of prognostic molecules and new targets for a multifaceted therapeutic approach to the disease.

**PP20. The effect of platelet-rich plasma on improving ovarian cell functionality: Reporting on data of an in vitro culture system of chemotherapy-induced ovarian insufficiency model.**

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Platelet-rich Plasma (PRP) intraovarian infusion has been suggested to enhance reproductive outcomes in women with ovarian insufficiency by improving ovarian microenvironment. However, due to limited data available regarding the effect of PRP on a molecular level, it is still considered experimental. This study aims to elucidate the physiological mechanisms of PRP, employing an in-vitro culture system mimicking ovarian insufficiency. Ovarian cells were cultured under conventional conditions and were treated for 72h with 4HC to induce chemotherapy-damage mimicking ovarian insufficiency. PRP was obtained from three healthy individuals (1.000.000platelets/ml). Following 72h, rFSH/rLH/PRP (rFSH/rLH 0.2 IU/ml) were added at varying PRP volumes of the initial preparation (50μL-100μL-200μL). Estradiol, VEGF, IGFII, AMH, and activin secretion, CYP19A1 expression and cell proliferation, as well as the levels of necrosis, apoptosis and senescence were analyzed. No difference was observed regarding estradiol, VEGF, IGFII levels. A significant difference was observed regarding AMH levels (50μL:370.67±39.19 vs 100μL:793.36±65.64 vs 200μL:820.44±297.78 vs control:238.71±100.51; p-value=0.01). Post-hoc analysis revealed differences between100μL group and control(p-value=0.04) and between 200L group and control(p-value=0.04). A significant difference was observed regarding activin (50μL:2.59±2.37 vs100μL:8.51±1.92 vs 200μL:17.41±8.30 vs control:18.16±4.26; p-value=0.04). Regarding cell proliferation, a significant difference was observed between 50μL group and control (226±50% vs 100±0%, p-value=0.04) and between 50μL group and 200μL group (226±50% vs 90±17%, p-value=0.001). Higher necrosis levels were observed in control compared to 50μL, 100μL, and 200μL groups (54.7% vs 42.6% vs 46.5% vs 42.78%, p-value<0.001). Apoptosis was lower in control, 100μL and 200μL groups compared to 50μL group (1.38%vs8.29%vs3.04%vs12.58%, p-value<0.001). Senescence was lower in control, 50μL and 200μL groups compared to 100μL group (0.1%vs5.38%vs5.24%vs14.54%, p-value<0.001). Higher CYP19A1 expression was observed in groups 50μL and 100μL compared to control. PRP seems to improve cellular functionality, vitality and proliferation. However, higher PRP concentration are associated with

increased apoptosis, suggesting a possible toxic effect, highlighting the need for further investigation on the optimal PRP dosage.

**PP21. Nutrition mediated decline of physical performance affects human functioning of Cretan elderly community dwellers.**

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Human functioning is a novel term that refers to the dynamic interaction of physiological health, lived health and contextual factors (environmental and personal that affect performance). According to WHO human functioning will supplement morbidity and mortality as a third health indicator. Here, we assessed human functioning in elderly insular community dwellers of Ierapetra, Crete.A cross-sectional study was done in residents of Ierapetra, Crete aged ≥65y. Data were collected via questionnaires including EQ-5D-5L (self-reported health level, SHL), Katz index (activities of daily living, ADLs) and Lawton index (instrumental activities of daily living, iADLs). Physical performance was assessed by 30s Sit to Stand test (leg power),6m Up & Go test (gait speed) and grip strength. Blood samples for biochemical and hematological testing were collected and analyzed. Serum markers were associated with physical performance and quality of life questionnaires using logistic or linear regression models adjusted for sex and age.164 individuals were included with median age 77y (10). Albumin (alb) levels were found to affect SHL (for each 1 g/dl alb increase, 17.49 increase in SHL, $p<0.006$). Leg power and gait speed mediated the effect of alb on SHL (indirect effect; for 1 g/dl increase in alb11.5 and 5.92 in SHL for leg power and gait speed, respectively). ADLs were negatively associated with white blood cell count (OR=0.83, CI 0.70-0.97) and creatinine (Or=0.37,CI 0.14-0.96) increase and positively associated with HDL (OR=1.03, CI 1.01-1.06) andAlb (OR=7.58, CI 1.96-30.69) increase. Alb and hemoglobin increase were also positively associated with iADLs (OR=24.48, CI 5.55-114.93 and OR=1.37, CI 1.07-1.76respectively). Human functioning was found to be affected in elderly insular Greeks through nutritional and physical performance decline. These results will be compared to data from Blue Zone elderly insular Greek dwellers.

**PP22. Inverse Correlation Between Type I Interferon Pathway Activation And Unstimulated Whole Salivary Flow In Sicca Patients**.

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Given the established role of interferon (IFN) pathway activation in Sjögren's disease (SjD) pathogenesis, we investigated whether demographic, clinicopathological, and laboratory characteristics, together with IFN-inducible gene expression, are associated with impaired salivary secretion in patients with sicca symptoms.

A total of 623 consecutive patients were evaluated at the Department of Physiology, Medical School of Athens, National and Kapodistrian University of Athens (NKUA), following informed consent. Comprehensive clinical and laboratory data were collected, including immune profiling, testing for chronic viral infections (HIV, HCV), chest X-ray, and minor salivary gland (MSG) biopsy. Expression of type I and type II IFN-inducible genes (IFIGs) was quantified by real-time PCR in 110 MSG biopsies, with parallel assessment in peripheral blood. In a subset of 10 patients with SjD (6 with normal and 4 with reduced unstimulated whole saliva [UWS]), a pilot bulk RNA-sequencing study was performed in addition to next-generation sequencing (NGS). Total RNA was extracted from peripheral blood leukocytes following quality control. Statistical analyses were conducted using SPSS v29.0, and bioinformatic analyses in R. Unstimulated whole salivary flow (UWSF) was inversely associated with type I and II IFN transcripts, clinical indices of dryness, anti-Ro/SSA and anti-La/SSB seropositivity, inflammatory markers, and MSG histopathological parameters, including focus score, Tarpley score, and fat score. Differential gene expression analysis revealed that genes upregulated in patients with low UWS were enriched for innate immunity–related pathways, including antiviral defense, type I IFN response, and regulation of IFN production.

Overall, these findings demonstrate a strong association between IFN pathway activation and salivary gland hypofunction, highlighting enhanced interferon signaling and antiviral immune responses in SjD patients with reduced salivary flow.

**References:** 1Mavragani et al: CMAJ 2014 Oct 21;186(15):E579-86

2Marketos et al: Clin Exp Rheumatol 2019; 37 (Suppl. 118): S185-S191

S191

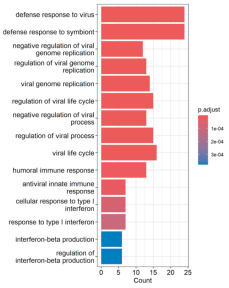


*Figure 1-Gene Ontology (GO) analysis of upregulated genes in patients with low versus normal unstimulated salivary flow rates.*

**PP23. Molecular physiology of aging and cancer: Impact of mutations on the function oflongevity-associated genes in exceptional responders.**

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Background: Exceptional responders are defined as cancer patients who exhibit long-term disease remission after therapy, yet the underlying mechanisms remain unclear. Aim: Given the association between aging and cancer, we explored the potential impact of mutations on the function of longevity-associated genes in a cohort of exceptional responders (ER). Methods: We used the Clinical and Genomic Data portal (GDC) to access the ER program. We filtered the cohort (84 cases) and analyzed mutation frequency across four genes (KL; Klotho, CISD2; CDGSH iron-sulfur domain 2, SIRT1 and SIRT6; Sirtuins) that have been associated with longevity. To estimate the impact of amino acid substitutions on protein structure and function, we retrieved the PolyPhen score (0.0–0.15: benign;0.15–0.85: possibly damaging; 0.85–1.0: probably damaging). Mutated genes were further inputted in the String v.12.0 server for network enrichment. Results: The primary cancer sites in the ER cohort were colon, bronchus, and lung. 19/84cases were tested for simple somatic mutations. 2/19 (10.53%), 1/19 (5.26%), and 1/19(5.26%) cases had mutations in SIRT1, SIRT6, and KL, respectively. The corresponding rates across the GDC portal were 0.82%, 0.37%, and 1.92%. All mutations in ER patients were identified as benign. The String database added 5 nodes to the KL-SIRT1-SIRT6network (NAD kinase 2; NADK2, ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2; BST1, Nicotinamide N-methyltransferase; NNMT, Ectonucleotide pyrophosphatase/phosphodiesterase family member 1; ENPP1, and NAD(P) transhydrogenase, mitochondrial; NNT). Nicotinate and nicotinamide metabolism, as well as metabolic pathways, were significantly enriched. Conclusions: The increased number of ER cases with benign variants in longevity genes, compared with cohorts across the GDC, suggests that SIRT1, SIRT6, and KL should be investigated as potential targets for cancer therapy through their regulation of physiological processes associated with NAD metabolism.

**PP24. Pericardial permeability is determined by the mesothelium and altered by Primary Cilium perturbing drugs.**

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The pericardium serves as a complex semi-permeable serous membrane that maintains the mechanical and biochemical microenvironment of the heart through the regulated transport of fluid and solutes. Despite its critical role in cardiac protection, the molecular pathways and physical forces like such as mesothelial cell integrity and hydraulic conductivity that govern its permeability remain insufficiently characterized in both physiological and pathological states. This study aimed to delineate the mechanisms of pericardial transport by investigating the role of mesothelial layer cohesion in the generation of transmembrane resistance ($R_{TM}$) using an ex vivo sheep pericardial tissue as a model. Additionally, we evaluated the effects of various pharmacological agents and chemical stressors on the permeability of the parietal pericardial membrane, including pericarditis treatment related drugs (colchicine and ibuprofen) as well as drugs that perturb the Primary Cilium [ammoniumsulfate (AS), chloral hydrate (CH) and lithium chloride (LC)]. R TM was measured continuously in an Ussing chamber before and after the addition of these substances to identify changes in ionic permeability.

Our results demonstrated that the mechanical or chemical denudation of mesothelial cells leads to a near total loss of the pericardial barrier function regarding electrolyte transport as evidenced by histology and R TM measurements. Furthermore, AS and LC induced a statistically significant decrease in the R TM indicating increased ionic flux. Conversely, ibuprofen induced a significant increase in R TM at 30 minutes post administration, suggesting a tightening of the mesothelial junctions or modulation of active transport pathways. These findings highlight the pivotal role of the mesothelium in regulating pericardial permeability, a significant role for the mesothelial Primary Cilium and suggest that pharmacological modulation of the mesothelial barrier could provide therapeutic opportunities in pericardial effusions.

**PP25. DNA replication licensing aberrations as a genetic trait of colorectal cancer.**

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Genomic instability, described as a state with high frequency of genetic mutations, is the driving force of cancer progression. Exhibited as persistent DNA damage, mitotic defects and chromosomal rearrangements, genomic instability is the combined effect of replication stress and deficient DNA damage response (DDR), both associated with oncogenes' activation. Intriguingly, several lines of evidence suggest that oncogene-induced replication stress is fuelled by aberrant licensing of DNA replication, which is a common feature of cancer cells. Replication licensing is a strictly regulated process that secures the timely and precise duplication of the genome in each cell cycle. Licensing takes place from late mitosis and through G1 phase and is accomplished by the recruitment of the licensing factors to the replication origins that are spread throughout the genome. Previous studies have shown that overexpression of licensing factors leads to aberrant licensing and a subsequent replication stress phenotype. Here, we focus on the overexpression of the licensing factor Cdt1. Interestingly, analysis of transcriptomics data derived from human cancers revealed that tumours that overexpress Cdt1 are associated with poor prognosis. Moreover, overexpression of Cdt1 in a mouse model of chemically induced colorectal cancer led to a more aggressive phenotype compared to the control condition1. Cdt1-overexpressing tumours indicated accumulation of DNA damage and increased nucleus staining, which are representative signs of replication stress. Further analysis in cancer cell lines confirmed the consequences of Cdt1 overexpression in genome stability. Intriguingly, we have observed that normal, non-transformed cells, will not exhibit genotoxic stress upon Cdt1 overexpression. These observations suggest that Cdt1 could potentially serve as a therapeutic target especially in colorectal cancer. Overall, our work highlights the role of impaired licensing, and especially Cdt1, in cancer progression and primes the landscape for developing novel therapeutic interventions.

1 Petropoulos, M. JPathol 2023

2 Zhu W, Canc Res 2009

**PP26. Physiological Remodeling of Tumor-Draining Lymph Nodes in Colorectal Cancer.**

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Colorectal cancer (CRC) is responsible for the second most cancer-associated deaths. Most CRC patients develop liver metastasis. The cancer cells first infiltrate the draining lymph nodes and then create metastatic sites at distant organs. Moreover, the lymphatic spreading of CRC is associated with poor prognosis and there are currently no treatments available to inhibit metastasis. The current CRC management guidelines are based on this model, however, the pathophysiological mechanisms underlying the lymphatic spreading and thus the liver metastasis are unknown. Tumor-draining lymph nodes(TDLNs) represent critical immune regulatory organs that integrate local signals and shape the anti-tumor responses. We hypothesized that primary CRC induces functional and immunological changes in TDLNs that contribute to the establishment of a permissive environment for liver metastasis. Using an orthotopic murine model of CRC with spontaneous liver metastasis formation, immune composition was analysed across different primary CRC locations. In vivo CD45 labelling allowed for the discrimination between circulating and resident immune cells. TDLNs were identified in vivo using indocyanine green (ICG) lymphatic mapping, allowing the discrimination between cancer-infiltrated (ICG+) and non-infiltrated(ICG-) TDLNs. TDLNs exhibited alterations in the composition of resident T cell population. TDLNs also demonstrated changes in modulation of interferon-γ producing T cell subsets. Usingf luorescent labelled cancer cells in flow cytometry analysis we identified that ICG+ TDLNs are indeed infiltrated, validating this approach for identifying the cancer-related TDLNs. Together, these findings demonstrate that primary CRC tumors induce early physiologicaland immunological remodeling of TDLNs T cell composition and IFNγ responses. These data identify tumor-draining lymph nodes as early immunologically altered sites during CRC progression.

**PP27. DCA addition to standard chemotherapeutics in pleural mesothelioma ameliorates 2Dbut3 D cell viability.**

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Pleural mesothelioma (PM) is an aggressive malignancy that is mainly resistant to chemotherapy. Cisplatin and Pemetrexed are the first line chemotherapeutics for MPM. Cancer cell metabolic reprogramming and specifically the dependency of cancer cells to anaerobic glycolysis, known as the Warburg effect, has been proposed as a mechanism supporting the resistance to chemotherapy. Here we investigated whether treatment with Dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase, enhances the effectiveness of chemotherapy with Cisplatin and Pemetrexed in PM cancer cell lines, using two-dimensional (2D) and three-dimensional(3D)in vitro models. Using the JL-1 cell line, an epithelioid mesothelioma cell line, we performed dose response experiments to determine the IC50 of DCA. Based on these results and cross referencing with existing literature, a concentration of 10mM DCA was selected for the functional in vitro cell assays. Cell viability showed that the combination of DCA with Cisplatin and Pemetrexed significantly reduced the cell viability compared to Cisplatin and Pemetrexed alone, indicating an additive effect of the metabolic inhibition. Similar trends were observed also with the use of 2-deoxy-glucose, an alternative inhibitor of glycolysis. In contrary, treatment with DCA did not affect the formation of 3D spheroids, using the hanging drop method, neither alone nor its combination with Cisplatin and Pemetrexed. This suggests that the metabolic inhibition affects mainly the cell viability and not the early stages of the 3D cellular aggregation. Our results suggest a dependence on the dimensional state of the PM cells during metabolic inhibition of the Warburg effect with DCA addition that enhances the response to standard chemotherapeutics in cell viability assays, without however affecting the early spheroid formation.

**PP28. Double-stranded RNA and anti–double-stranded RNA natural antibody profiles in patients with parapneumonic and malignant pleural effusions.**

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Parapneumonic and malignant pleural effusions are common exudative clinical conditions with overlapping clinical features, complicating diagnosis. Immune responses within the pleural space remain poorly understood, especially in pleural effusions. In this context, the role of double-stranded RNA (dsRNA), a key innate immune stimulus, and natural antibody (NAb) responses in pleural immunity remains largely underexplored. Here, dsRNA and NAbs against dsRNA of IgM, IgA, and IgG class were measured in pleural effusions from patients with parapneumonic (PPE, n=28) and malignant effusions (MPE, n=27) using in-house ELISAs. Potential associations with established biochemical markers were also assessed to determine whether these measures could distinguish PPE from MPE in diagnosis. Significantly higher dsRNA levels and IgA anti-dsRNA antibodies were found in PPEs, while dsRNA levels correlated strongly with IgM anti-dsRNA antibodies across all samples. A logistic regression model combining dsRNA and anti-dsRNA levels differentiated PPEs from MPEs with high accuracy (AUC = 0.914). These findings reveal distinct dsRNA and NAb profiles in pleural effusions, suggesting a role for IgM NAbs in dsRNA homeostasis and for IgA NAbs in local infection-related immune responses. This study provides new insights into pleural immune mechanisms and highlights the potential of dsRNA-associated markers as biomarkers for effusion classification.

**PP29. Differential effect of biphasic mesothelioma cell conditioned media and patient derived malignant pleural effusion fluid on the permeability of normal mesothelial cell monolayers.**

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Aim Malignant pleural effusion (MPE) is characterized by overwhelming buildup of fluid within the pleural space secondary to metastatic disease or primary pleural malignancy, predominantly mesothelioma. Despite the key role of pleural hyper-permeability, it remains unexplored whether the mesothelial cell barrier that ubiquitously lines the pleural surface participates in MPE formation. Given the dynamic effects of various stimuli such as growth factors and cytokines on mesothelial layer integrity, we sought to determine whether biphasic malignant mesothelioma cells MSTO-211H secrete mediators that impinge on Met5A monolayers in a paracrine manner.

Methods We evaluated transmesothelial electrical resistance (RTM), an inverse surrogate of membrane permeability, 10 kDA FITC-labeled dextran flux, reflective of paracellular permeability and mRNA expression of tight junction protein-1, occludin, claudin-1, claudin-2, claudin-3,claudin-5 and claudin-15, components of the tight junction, the molecular correlate of paracellular permeability. MeT5A cells were grown on Snapwell filters until confluence and were mounted on Ussing chambers for RTM measurement every 15min for 2h.

Results No significant differences were found between chronically exposed monolayers (24hculture in MSTO-211H conditioned media), acutely exposed monolayers (apical addition of MSTO-211H conditioned media at t=0) and control experiments (apical addition ofMeT5A conditioned media at t=0). MeT5A cells were grown on Transwell filters until confluence and were exposed for 24h to malignant pleural effusion fluid. Baseline and24h RTM values were recorded with an STX2 chopstick electrode. A significant reduction in RTM was observed compared to controls. No significant differences in terms of 10 kDa dextran flux and mRNA of tight junction components were observed.

Conclusions The findings suggest that prolonged exposure to patient malignant pleural effusion fluid decreases RTM of mesothelial monolayers.

*This work was supported by a grant from the Hellenic Thoracic Societ

**PP30. Investigation of Slit2 – Robo4 pathway involvement in malignant and parapneumonicpleural effusions.**

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Background: Pleural effusion formation is determined by altered pleural vascular and mesothelial permeability during inflammatory and malignant conditions. The Slit – Robo pathway, especially the Slit2 – Robo4 axis has been implicated in endothelial barrier regulation. However, it is not known whether it has a role in pleural effusion pathophysiology. Aim and objectives: The aim of this study was to investigate whether the Slit2 – Robo4 pathway is involved in pleural effusion formation. We hypothesized that pleural fluid concentrations of solubleSlit2 and Robo4 differ between malignant and parapneumonic pleural fluids and that they are associated with biochemical markers of pleural vascular permeability. Methods: Pleural fluid samples were collected from 42 patients, treated at the Department of Respiratory Medicine of the University Hospital of Larissa. Twenty-one (21) patients had parapneumonic pleural effusions, further classified as uncomplicated (n=9) or complicated(n=12), and 21 patients had malignant pleural effusions. Slit2 and Robo4 levels we requantified using enzyme – linked immunosorbent assay (ELISA). Comparisons between pleural effusions subtypes were performed, and correlations between Slit2, Robo4 and pleural fluid biochemical parameters were assessed. Results: Soluble Slit2 and Robo4 were detectable in all pleural fluid samples. No statistically significant differences in Slit2 or Robo4 concentrations were observed between malignant and parapneumonic pleural effusions 1.7 (1.2 – 2.75) vs 2 (1.25 – 2.8), p=0.68 and 50.4(42.45 – 63.8) vs 54.1 (41.85 – 71.8), p=0.86, respectively, neither between uncomplicated and complicated parapneumonic pleural effusions 1.7 (1.25 – 2.45) vs2.2 (1.05 – 3.4), p=0.43 and 54.08±21.45 vs 61.49 ±24.34, p=0.48 respectively. Also, there were no differences in their ratios in pleural effusions. In complicated pleural effusions, Slit-2 concentrations were inversely correlated with pleural fluid albumin levels (r= -0.62, 95% CI -0.88 to – 0.07; P=0.03). Conclusions: Slit2 and Robo4 levels did not distinguish pleural effusion.

**PP31. Exploring the interplay of HIF-2α and ALDH1As in neural crest cells differentiation: implications for neuroblastoma development**

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Neuroblastoma (NB) is an infant malignancy originating from the multipotent neural crest cells (NCCs) during fetal development. Irregular NCC differentiation is regarded as key driver of NB onset and tumors enriched in undifferentiated, NCC-like cancer stem cells (CSCs) are associated with worse prognosis. Here, we investigated the mechanisms underlying this dysregulation by focusing on the interplay between hypoxia-inducible factor 2 alpha (HIF-2α) and aldehyde dehydrogenase 1A subfamily (ALDH1As), two factors implicated in stem cell differentiation and CSC phenotype. HIF-2α mediates cellular adaptation to hypoxia; its de-regulation is associated with abnormal NCC migration/differentiation, and NBs with HIF-2a positive cells in oxygenated areas have poor prognosis. ALDH1As contribute to differentiation via retinoic acid synthesis and are established as CSC markers in several malignancies. Emerging evidence suggests a positive bidirectional network between ALDH1As and HIF-2α. For examining whether HIF-2α regulates ALDH1As, we performed real-time PCR on NCCs from CRISPR/Cas9-mediated HIF-2α knockout chick embryos generated in previous studies. Chick embryos were selected due to their amenability to genetic manipulation and long-term embryogenesis studies. We detected altered ALDH1A expression after CRISPR/Cas9 mediated knockout of HIF-2a in chick embryos *in vivo*, as well as in established crestospheres (*in vitro* maintained NCCs retaining self-renewal/multipotency) compared with controls. Among ALDH1A members, ALDH1A2 showed the most robust and consistent downregulation among embryos and crestospheres and was selected for further investigation. Accordingly, we generated a single-plasmid CRISPR/Cas9 construct targeting ALDH1A2, based on the approach by Gandhi et al. The HH–gRNA–HDV cassette was first constructed in the shuttle vector and subsequently subcloned into the pCAG>nls-Cas9-nls-2A-Citrine plasmid. Sequencing confirmed the successful generation of three constructs. These constructs will be used to

knockdown ALDH1A2 in chick embryos, at the pre-migratory NCC stage, to assess HIF-2α expression, effects on NCC migration/differentiation, embryogenic development, and early events relevant to NB initiation or priming.

**PP32. Metabolic Reprogramming Driven by ALDH1B1 Overexpression in Human Colon Cancer Cells**

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Aldehyde dehydrogenases (ALDHs) are NAD(P)$^+$-dependent enzymes that catalyze the oxidation of endogenous and exogenous aldehydes to their corresponding carboxylic acids. In humans, ALDHs exhibit a wide range of substrate specificity, while participating in diverse biological processes, including antioxidant defense and cellular differentiation. Moreover, several ALDH isoforms are established markers of cancer stem cells (CSCs). ALDH1B1 is a mitochondrial enzyme, whose main substrates are retinaldehyde, 4-hydroxynonenal, and acetaldehyde. Previous studies have demonstrated its pathophysiological role in human colon cancer in respect to cell morphology, cell cycle progression, chemoresistance, epithelial-mesenchymal transition, and DNA damage response. This study focuses on the role of ALDH1B1 in cancer cell metabolism, using two isogenic HT29 colon cancer cell lines differing in ALDH1B1 expression. Total mRNA was isolated from biological triplicates of each cell line and subjected to transcriptomic sequencing and downstream analysis. Comparative profiling revealed 2,405 significantly differentially expressed genes in ALDH1B1-overexpressing (ALDH1B1$^+$) cells relative to mock controls, of which 463 were associated with metabolic pathways. Notably, several genes encoding components of electron transport chain were differentially expressed. ALDH1B1$^+$ cells exhibited downregulation of lactate dehydrogenase A, a key enzyme of anaerobic glycolysis associated with the Warburg effect, alongside upregulation of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme in the pentose phosphate pathway. Additionally, several tricarboxylic acid (TCA) cycle genes were upregulated, indicating a functional TCA cycle and controlled production of NADH and biosynthetic intermediates. These metabolic features are consistent with the non-canonical TCA cycle described in CSCs, supporting anaplerosis, redox homeostasis and self-renewal. Furthermore, carbonic anhydrases CA2 and CA9 were significantly upregulated, suggesting altered intracellular pH regulation and tumor microenvironment acidification. Intracellular ATP quantification demonstrated two-fold lower ATP levels in ALDH1B1$^+$ cells, possibly reflecting increased ATP consumption. Overall, this study highlights potential metabolic adaptations driven by ALDH1B1 overexpression that may promote tumor survival and growth.

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