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Species-specific effects of mitochondria-rich extracellular vesicles from human, canine, and porcine plasma and serum on cardiomyocyte proliferation

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Mitochondria are increasingly recognized as regulators of cardiomyocyte proliferation, a heart repair and regeneration target. However, despite extensive research, no current treatments effectively promote cardiomyocyte proliferation by directly targeting their mitochondrial content. The ability of extracellular vesicles (EVs) to deliver healthy mitochondria (mtEVs) to cardiomyocytes suggests a potential role in stimulating these cells to re-enter the cell cycle. However, human EVs are restricted in large-scale clinical applications. Therefore, we focused on the identification of alternative xenogeneic source of mtEVs from animals similar to humans in terms of their cardiovascular physiology. The aim of the study was to explore potential species-specific effects on cardiomyocyte proliferation of mtEVs from human, canine and porcine plasma and serum. EVs were isolated from venous plasma or serum of healthy young adult males humans (n=6), dogs (n=6) and minipigs (n=6) through serial centrifugation. Transmission electron microscopy (TEM) was used to evaluate the integrity of the EVs and to detect intact mitochondria. Circulating mtEVs levels were measured by using CytoFLEX flow cytometer and mean fluorescence intensity in the PC5.5 emission region was analysed with the CytExpert software. The viability of vesicular mitochondria and their internalization in rat cardiomyocyte H9C2 were assessed by confocal microscopy after labeling with MitoBrilliant™ 646. H9C2 (60000/well for 6-well; 2000/well for 96-well) were seeded for 24 hours (h) and were treated for 60h with each type of EVs pool (respectively, 300ul and 10μL). The evaluation of cell density (cell counting) and cell viability (MTT assay) was performed after treatment for 60h. Flow cytometry revealed higher mtEVs levels in dog plasma EVs compared to serum (p=0.02). No statistical differences were found between plasma and serum mtEVs in human and pig samples. Dog plasma mtEVs concentrations were higher than pig plasma (p=0.004) but comparable to human plasma. H9C2 cells exhibited similar uptake of plasma or serum mtEVs from both species. The number of viable H9C2 cells treated with dog or human plasma EVs pool was higher than untreated cells (P=0.0005 and P=0.003, respectively). However, no significant increase was observed with porcine plasma EVs pool. In conclusion, both canine and human plasma have comparable levels of mtEVs. These mtEVs stimulate the growth of cardiomyocytes to a similar extent. Our preliminary results suggest that dogs are suitable donors of xenogenic mtEVs for cardiac regeneration therapy.

Relevance to Conditioning Medicine: This study explores mtEVs from different species to promote cardiomyocyte proliferation, relevant to Conditioning Medicine's focus on cellular response to stress and injury and developing therapeutic approaches for heart repair.

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Link between estrogen intake and cardiac arrhythmias in long QT syndrome type 2

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Long QT syndrome type 2 (LQT2) is a genetic disorder characterized by abnormalities in the cardiac repolarization, leading to an increased risk of life-threatening arrhythmias. Recent evidence indicates that the administration of exogenous estradiol (E2) can act as a trigger of malignant cardiac arrhythmias in LQT2 patients. The aim of the project is to

study whether acute exposure to E2 causes cellular effects compatible with the facilitation of arrhythmias in the syndrome and to investigate the mechanisms. The study was conducted on cardiomyocytes differentiated from pluripotent stem cells (hiPSC-CMs) derived from a LQT2 patient (MUT) with arrhythmias induced by estrogen-based therapy and from a healthy donor (WT). Electrophysiological experiments were performed using the whole-cell patch-clamp technique. MUT and WT cells were studied in control conditions or after pre-incubation with E2 (10 nM) for 10 minutes. In spontaneously beating cells, the action potential duration (APD) was rate-corrected with Fridericia's formula, thus obtaining c_APD values. The I/V relationship, the steady-state activation and inactivation curves, the Ca^{2+}-dependent inactivation (CDI) and the recovery from inactivation of ICaL were studied. In MUT hiPSC-CMs, c_APD values were significantly increased ($p<0.0001$) compared to WT. Pre-incubation with E2 significantly shortened all c_APD values in MUT cells only. Despite APD shortening, E2 tended to increase the incidence of EADs. As all the observed E2 effects might result from changes in ICaL, the latter was also evaluated. The treatment with E2 shifted the ICaL activation curve towards positive potentials, while leaving the inactivation curve unchanged, thus narrowing the membrane potential range for steady-state current ("ICaL window"). Finally, E2 accelerated the kinetics of recovery from inactivation of ICaL. The remaining ICaL properties, including CDI, were unaffected. Notably, E2 effects were not observed in WT cells. In conclusion, the hiPSC-CMs model recapitulates the LQT2 clinical phenotype. Shortening of c_APD by E2 may result from the reduction of "ICaL window" which is essential for the current contribution to the action potential plateau. The faster recovery from inactivation of ICaL during the action potential plateau phase might account for EADs facilitation despite APD shortening.

Relevance to Conditioning Medicine: This study investigates how acute exposure to E2 affects cardiomyocytes in LQT2, contributing to arrhythmia risk. Understanding these cellular mechanisms aligns with Conditioning Medicine's focus on cellular responses to stress and injury, aiming to develop therapeutic strategies for managing and preventing arrhythmias.

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Striatin knock out induces a gain of function of INa and impaired Ca^{2+} handling in mESC-derived cardiomyocytes

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Striatin (Strn) is a scaffold protein expressed in cardiomyocytes (CMs) and alteration of its expression are described in various cardiac diseases. However, the alteration underlying its pathogenicity have been poorly investigated. We studied the role(s) of cardiac Strn gene (STRN) by comparing the functional properties of CMs, generated from Strn-KO and isogenic WT mouse embryonic stem cell lines. The spontaneous beating rate of Strn-KO CMs was faster than WT cells, and this correlated with a larger fast INa conductance and no changes in If. Paced (2–8 Hz) Strn-KO CMs showed prolonged action potential (AP) duration in comparison with WT CMs and this was not associated with changes in ICaL and IKr. Motion video tracking analysis highlighted an altered contraction in Strn-KO CMs; this was associated with a global increase in intracellular Ca^{2+}, caused by an enhanced late Na^+ current density (INaL) and a reduced Na^+/Ca^{2+} exchanger (NCX) activity and expression. Immunofluorescence analysis confirmed the higher Na^+ channel expression and a more dynamic microtubule network in Strn-KO CMs than in WT. Indeed, incubation of Strn-KO CMs with the microtubule stabilizer taxol, induced a rescue (downregulation) of INa conductance toward WT levels. Loss of STRN alters CMs electrical and contractile profiles and affects cell functionality by a disarrangement of Strn-related multi-protein complexes. This leads to impaired microtubules dynamics and Na^+ channels trafficking to the plasma membrane, causing a global Na^+ and Ca^{2+} enhancement.

Relevance to Conditioning Medicine: This study examines how the loss of Strn affects cardiomyocyte function, including electrical and contractile properties. By elucidating the cellular mechanisms underlying these changes, the research contributes to Conditioning Medicine's goal of understanding stress responses and developing therapeutic strategies for cardiac diseases.

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Silicon carbide nanowires enhance impulse propagation and resynchronization in atrial cells: in-vitro insights for atrial fibrillation management

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Atrial Fibrillation (AF) is a potentially life-threatening cardiac arrhythmia affecting millions of individuals annually. Current therapeutic approaches involve local tissue atrial ablation and electrophysiological investigations of the atrial chamber. However, these strategies can inadvertently lead to tissue necrosis and fibrosis post-ablation, disrupting the normal pattern of impulse propagation. This breakdown may contribute to the progression from paroxysmal to persistent AF. Recently, our research has explored the innovative use of Silicon Carbide Conductive Nanowires (SiC-NWs) to facilitate propagation in rat ventricular infarct tissue resulting from cryoablation. By injecting SiC-NWs into the unexcitable infarct area, we observed acute ventricular resynchronization, prompting further investigation into their potential for terminating AF. We present our in-vitro investigation toward the ability of SiC-NWs to support impulse propagation in atrial cells. These cells were obtained through the differentiation of human Rockefeller University Embryonic Stem Cells (RuES) into atrial monolayers and 3D atrial cardioids. RuES cells were subjected to differentiation protocols utilizing the STEMdiff Atrial Cardiomyocyte Differentiation Kit. Following differentiation, cells were cultured and purified. SiC-NWs, at 50μg/ml, were administered to unsynchronized beating monolayers of atrial cells. SiC-NWs were deposited

within the gap between physically separated monolayers to assess their effect on impulse synchronization. Additionally, SiC-NWs were incorporated during the formation of 3D cardioids. Cardiac mechanics were assessed using the LOKI platform, a customized technology capable of acquiring high-res. videos in high-throughput screening mode. SiC-NWs exhibit biocompatibility and do not disrupt the normal electromechanical coupling of the 2D and 3D preparations. After 24h, we observed the resynchronization of two adjacent but previously separate monolayers of spontaneously beating atrial cells, with SiC-NWs facilitating impulse propagation within the gap. These cells exhibited an average beat duration of ca. 300 ms and contraction time (234 ± 88 ms), relaxation time (214 ± 84 ms) and beat duration of 565 ± 74 ms demonstrating significant improvement compared to untreated preparations where resynchronization was not achieved. Interestingly, in 3D cardioids with embedded SiC-NWs, we did not observe significant changes in propagation and mechanics, suggesting that in normal tissue, SiC-NWs do not alter physiological parameters. These findings provide promising insights toward the use of SiC-NWs to facilitate the resynchronization of activity in atrial cells. Should these results translate to in-vivo settings, potentially leading to the adoption of nanotechnology-based interventions in clinical practice for managing AF. Further research is warranted to validate and expand upon these initial observations.

Relevance to Conditioning Medicine: This study investigates whether the use of SiC-NWs to facilitate impulse propagation and resynchronization in atrial cells could improve outcomes for AF patients. This aligns with Conditioning Medicine's focus on innovative therapeutic strategies to address cardiac stress and injury, aiming to enhance cardiac repair and function.

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Impact of particulate matter on cardiac spheroids: implications for foetal heart development

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Particulate Matter (PM) is a major component of air pollution and is well known to trigger non-communicable diseases. PM can be found in various human tissues of individuals living in polluted areas. It can also pass through the placenta and reach the embryonic heart. However, it is not known whether PM can interfere with and deteriorate the heart performance of unborn children, as it is difficult to constantly monitor the foetal heart. Spheroids, which are aggregates of cells forming 3D structures, offer a promising approach for directly studying complex organs and their interactions with harmful substances. In this study, we examined the effect of PM2.5 on cardioids derived from embryonic stem cells as a model for the developing heart. Cardiac spheroids were generated from differentiated Rockefeller University Embryonic Stem Cell Lines (RUES). Cells were thawed in E8 medium with 1% ROCK inhibitor. The following day, the medium was replaced with E8, which was changed every two days until reaching 60-70% confluence. Differentiation into cardiomyocytes was performed using the PSC Cardiomyocyte Differentiation Kit. Beating began after 12-14 days. Cells were then dissociated and filtered using magnetic columns after mixing with magnetic antibodies to remove undifferentiated cells. The eluted cardiomyocytes were seeded in an ultra-low attachment 96-well plate at a density of 50,000 cells to form spheroids. Zeta potential was determined, and cytokines were quantified using ELISA. Real-time PCR was performed with the QuantStudio 3 Real-Time PCR System. Calcium transients were obtained via an epifluorescence microscope equipped with the Kinetix 22 camera at 1,000 fps. Contractility was assessed with the same microscope equipped with a Basler camera at 150 fps. Zeta potential analysis revealed a negative charge in all the physiological media containing PM. This negative environment promotes the incorporation of PM into the spheroid, as observed under the microscope. Calcium transient analysis revealed a significant reduction in the time of Ca^{2+} transient reuptake, accompanied by a significant decrease in contractility (time of relaxation), indicating that PM significantly impacts the calcium reuptake machinery during organoid development. Molecular analyses showed a slight response of genes involved in calcium transport, while oxidative stress genes (HMOX1, SOD1, and SOD2) were upregulated in spheroids treated with PM2.5 at concentrations of 10 µg/ml and 20 µg/ml at 48 and 192 hours. Additionally, CACNA1C, RyR2, and SERCA2A, which regulate calcium reuptake inside the sarcoplasmic reticulum, were also affected by PM. These preliminary results align with the analysis of IL-6 and IL-8 levels in the same spheroids, suggesting strong oxidative stress induced by PM in cardioids. These data suggest a potential role of PM in crossing the placenta, indicating possible adverse effects on foetal heart development.

Relevance to Conditioning Medicine: This study aligns with Conditioning Medicine's focus on understanding the cellular response to environmental stressors and injury, aiming to develop therapeutic strategies to protect and enhance heart health.

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Investigating molecular mechanisms of high glucose-induced senescence in H9c2 cells: an in vitro study

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High glucose (HG) poses a significant risk for cardiovascular function decline, particularly in conditions such as diabetic cardiomyopathy (DCM), exacerbating cardiac ischemia/reperfusion (I/R) injury. Cellular senescence, entailing irreversible cell cycle arrest, is pivotal in cardiovascular diseases such as atherosclerosis, myocardial infarction, and cardiac fibrosis. In this study, we aim to investigate the molecular mechanisms underlying HG-induced senescence in cardiomyocytes, with a focus on NLRP3 inflammasome and selenoproteins involvement. For this purpose, we established an in vitro model using rat embryonic cardiomyocytes (H9c2) exposed to varying glucose levels. H9c2 cells were cultured with glucose concentrations ranging from 5.5 mM to 400 mM for 24 and 48 hours to assess metabolic stress levels indicative of senescence. Cell viability and proliferation were evaluated using MTT assay, while spectrophotometric analysis determined reactive oxygen species (ROS) levels as a marker of oxidative stress. We assessed senescence-associated β-galactosidase (SA-β-gal) activity, a key senescence marker, using an absorbance-revealing kit, with the SA-β- gal/MTT ratio serving as a senescence index. Evaluation of other markers is currently underway. Our preliminary data show an increased cell death (MTT reduction) at glucose concentrations above

100 mM (HG) accompanied by an elevated SA-β-gal/MTT ratio across all tested glucose concentrations (50 mM, 75 mM, 100 mM, and 400 mM) compared to controls at 48 hours (p values <0.0001 to 0.0006). Notably, cell death and the SA-β-gal/MTT ratio were markedly elevated at 400 mM glucose, indicating a correlation between glucose concentration and cell death. Preliminary testing suggested oxidative stress, warranting further investigation into underlying mechanisms. In conclusion, HG induces senescence in H9c2 cells, characterized by morphological alterations, oxidative stress, and SA-β-Gal overexpression. Notably, very HG concentrations (>100 mM) induce cell death, contributing to cardiotoxicity. Further studies are needed to elucidate the intricate relationship between oxidative stress, sterile inflammation, and senescence. Our ongoing experiments aim to deepen our understanding by exploring cellular morphology changes through immunofluorescence microscopy and investigating senescence signaling pathways and associated markers to enhance cardioprotection. Additionally, we are examining the role of NLRP3 and selenoproteins in the presence of HG levels.

Relevance to Conditioning Medicine: This investigation on senescence-related stress on cardiomyocytes under hyperglycemia aligns with Conditioning Medicine's focus on understanding stress and injury mechanisms for new therapeutic strategies.

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Interleukin 1β inhibition attenuates doxorubicin induced cardiotoxicity

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Anthracyclines, such as Doxorubicin (DOX), are effective anti-cancer drugs whose use is limited by cumulative dose-dependent cardiotoxicity involving cellular interplay and mechanisms of oxidative stress, apoptosis, and inflammation. In particular, DOX induces the release of proinflammatory cytokines, and several treatments are cardioprotective by reducing TNF-α and IL-1β levels. As IL-1β driven inflammation is an active player in the development of cardiac dysfunctions, we sought to evaluate the protective effect of IL-1β inhibition on DOX-induced cardiotoxicity. Methods: we exposed C57Bl wild-type mice to DOX chronic infusion (IP injection of 2.5 mg/kg, 3 times per week, for 2 weeks). Starting from the second week of DOX treatment, we blocked IL-1β by 01BSUR infusion (IP injection of 10 mg/kg for 1 time per week, for 3 weeks). After 1 month from the first DOX infusion, cardiac function was evaluated by echocardiography. Cardiac damage and inflammation were then assessed by ex-vivo biochemical studies. As expected, DOX induces a significant reduction of cardiac ejection and shortening fractions (EF and FS), alongside the increase of IL-1β circulating levels. The treatment with 01BSUR is effective in preventing DOX-induced cardiac EF and FS collapse. Accordingly, 01BSUR significantly attenuates cardiac p53 accumulation and ANP gene transcription induced by DOX. NFκB activation, a key player in inflammatory and oxidative stress mechanisms, was reduced in the heart of DOX + 01BSUR- treated mice compared with the DOX-treated group. In line, the transcription levels of IL-6, a direct product of IL-1β receptor/NFκB axis activation, were significantly lower in DOX + 01BSUR than in the group exposed to DOX alone. IL-1β signaling activation is involved in DOX-induced cardiac inflammation and damage. The inhibition of IL-1β signaling by 01BSUR is an effective cardioprotective strategy to counteract DOX cardiotoxicity.

Relevance to Conditioning Medicine: This study demonstrates that inhibiting IL-1β can protect against DOX-induced cardiotoxicity, highlighting the role of inflammatory signaling in cardiac injury. It aligns with Conditioning Medicine's focus on understanding and mitigating cellular responses to stress and injury, aiming to develop therapeutic strategies for cardioprotection in cancer treatment.

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MELAS syndrome under the microscope: electrophysiology and mechanics of hiPSCs-based cardioids

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MELAS syndrome (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like Episodes) is associated with cardiac conduction defects and hypertrophy. To further study the cardiac aspect of such complex disease, human induced pluripotent stem cells (hiPSCs) offer a unique substrate when differentiated into cardiomyocytes, preserving highly personalized phenotype. We adopted a highly adaptable in-vitro model, 3D cardioids, to explore potential impairments in electromechanical coupling of cells as well as calcium machinery and ATP expression. We utilized hiPSCs carrying the MELAS mutation and their respective isogenic controls differentiated into cardiomyocytes, cultured in 3D cardioids. Electromechanical coupling was assessed using laser-poration technology combined with video kinematics evaluation under various stimulation patterns. Moreover, CellTiter-Glo™ Luminescent Cell Viability assay was performed to quantify the ATP content in both classes. Finally, Fluo-4AM marker was used to determine eventual calcium flow differences. Dimensionally, the diseased spheroids appeared bigger (403.1 ± 12.31 μm *vs.* 436.6 ± 12.15 μm). From the mechanical analysis, MELAS samples exhibited slightly higher beating frequency in spontaneous beating regimen (0.554 ± 0.1068 Hz control *vs.* 0.6441 ± 0.2882 Hz MELAS). Additionally, the MELAS samples have significantly shorter beat duration (0.629 ± 0.067 s *vs.* 0.483 ± 0.056 s) as well as reduced upstroke contraction phase (0.2728 ± 0.023 s *vs.* 0.255 ± 0.014 s). Laser-poration technology, combined with MEA chips, revealed a significant modulation of action potential duration (0.308 ± 0.054 s *vs.* 0.195 ± 0.004 s). Quantification of electromechanical delay did not evidence any impairment in the diseased cell line. Luminescence analysis of ATP concentration revealed to be significantly lower in control samples compared to the pathological one (0.298 ± 0.029 μM *vs.* 0.5193 ± 0.135 μM). Ultimately, calcium analysis confirmed the overall faster kinematics of the MELAS syndrome spheroids, with all the parameters reported to be shortened. Specifically, a statistically significant decrease in time of calcium reuptake was highlighted (τfall050, 0.0277 ± 0.00131 ms *vs.* 0.02362 ± 0.0019 ms). This study further delves into MELAS hiPSCs differentiated into cardiomyocytes. The novel characterization offered by

Intracell® system allowed to characterize simultaneously the electrophysiology and the mechanics of the spheroids, with contact-free approaches. Moreover, these data combined to the dimensionality of the samples may suggest an enhanced conductivity of the diseased model, leaving open questions which will require nanoscale approaches to further characterize the differences. In conclusion, the introduced pipeline could be applied in translational studies as possible pharmacological effects on diseased samples.

Relevance to Conditioning Medicine: This study uses 3D cardioids derived from hiPSCs to explore electromechanical and metabolic dysfunctions in MELAS syndrome, focusing on calcium handling and ATP production. It aligns with Conditioning Medicine's goal of understanding cellular responses to stress and injury and developing therapeutic strategies to improve cardiac function in mitochondrial disorders.

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Sex-specific effects of estrogen and barley β-glucan on doxorubicin-induced endothelial injury

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Doxorubicin (DOXO) is a potent chemotherapeutic drug, but its use is limited by cardiotoxicity. The endothelium is a critical target for preventing and treating DOXO-induced cardiomyopathy. However, current understanding lacks investigation into sex-based differences in how DOXO injures the endothelium. This knowledge gap hinders the development of effective personalized treatment strategies. Antioxidants have shown promise in mitigating DOXO-induced endothelial injury, but their efficacy in a sex-specific context remains unexplored. Our study aims to assess the potential of barley (1-3) β-D-Glucan (BBG), a natural water-soluble polysaccharide with antioxidant properties, to protect endothelial cells against DOXO injury, specifically considering sex-based differences. Female and male Human Umbilical Vein Endothelial Cells (F-HUVECs; M-HUVECs) were exposed to DOXO (200nM) in the presence or absence of 5nM 17 β-estradiol. All groups were then treated with or without 3% (w/v) BBG for 72 hours. First, we found that DOXO caused more oxidative stress and senescence in M-HUVECs compared to F-HUVECs ($p<0.05$). This was measured by a dihydroethidium assay for oxidative stress and a β-galactosidase assay for senescence. Interestingly, even though this sex difference remained, 17β-estradiol treatment significantly increased cell proliferation and reduced DOXO-induced oxidative damage in both M-HUVECs and F-HUVECs. These findings suggest that 17β-estradiol might protect HUVECs from DOXO damage by preserving mitochondrial function, regardless of sex chromosomes. Indeed, treating the cells with 100uM 5-hydroxydecanoate, a specific inhibitor of mitochondrial ATP-sensitive potassium (K^+) channels (mitoKATP), significantly hampered the protective effect of 17β-estradiol against DOXO-induced injury in HUVECs from both sexes. Finally, MTT Cell Proliferation Assay revealed that BBG has a more pronounced stimulatory impact on M-HUVECs compared to F-HUVECs ($p<0.05$). However, this difference disappeared with 17β-estradiol treatment. Surprisingly, BBG did not provide additional endothelial protection against DOXO-induced injury, regardless of sex or estradiol treatment. Our preliminary data suggests that M-HUVECs are more susceptible to doxorubicin-induced premature senescence compared to F-HUVECs regardless hormonal influence. 17β-estradiol exerts greater protective effects against doxorubicin through regulation of mitoKATP in F-HUVECs. Supplementation with 3% BBG did not significantly improve endothelial protection from DOXO-induced injury compared to 17β-estradiol in either male or female human endothelial cells.

Relevance to Conditioning Medicine: This study examines how BBG and 17β-estradiol may protect endothelial cells from DOXO injury, with attention to sex-based differences. Understanding these mechanisms is vital for personalized treatment strategies to counter DOXO-induced cardiotoxicity, aligning with Conditioning Medicine's goal of addressing chemotherapy's impact on cardiovascular health, including sex-specific responses and antioxidant interventions.

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Serum lipoprotein dysfunction is a major determinant and independent clinical marker of coronary atherosclerosis and high cardiovascular risk in patients with rheumatoid arthritis

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Autoimmune rheumatic diseases, such as rheumatoid arthritis (RA), are associated with particularly high cardiovascular risk (CVR). The complexity of proatherogenic mechanisms makes the definition of single patient CVR very difficult, thus contributing to non-optimal prevention and treatment. 141 patients with RA from the PROspecTive Evaluation of Latent Coronary ATherosclerosis in RheumatoidArthritis (PROTECT- RA) cohort were studied prospectively for incident cardiovascular events over 6.0±2.4 years. Macrophage cholesterol loading capacity (CLC) of serum and cell ABCG1 or ABCA1 cholesterol efflux capacity (ABCG1-CEC or ABCA1-CEC, respectively) of HDL were measured at baseline and at follow up, together with a panel of laboratory parameters and concurrently with coronary computed tomography angiography (at follow up n=99). Serum CLC was measured fluorimetrically as intracellular cholesterol uptake in THP-1 macrophages after incubation with whole patient serum. HDL-CEC was determined radiometrically as the percent of cell cholesterol effluxed upon patient HDL incubation, in cell models validated for specific ABGC1-CEC or ABCA1-CEC measurement. Baseline CLC associated with incident cardiovascular event risk in RA patients after adjusting for Atherosclerotic Cardiovascular Disease (ASCVD) risk score (HR 1.76, [95%CI 1.16-2.67] per 1 SD higher CLC, $p=0.008$), and to high risk coronary plaque burden in biologic disease modifying drugs (bDMARD) nonusers ($p<0.001$). Major determinants of serum CLC were oxidized LDL (measured as oxidized phospholipids on apoB100), anti-oxidized LDL IgG and proprotein convertase subtilisin/kexin type 9 (PCSK9). In patients with low C-reactive protein (CRP) (≤ median), baseline ABCG1-CEC associated inversely with CVR (HR 0.47 per 1 SD higher ABCG1-CEC, $p=0.05$). It also negatively associated with extensive coronary atherosclerosis (HR 0.50, $p=0.017$), high-risk plaque burden (HR 0.063, $p=0.013$) and when both baseline and time-averaged CRP was low, also with plaque progression in time (p-for-

interaction =0.001 and 0.021 respectively). ABCA1-CEC associated with fewer calcified plaques at baseline (IRR 0.52 [95% CI 0.32–0.83], p =0.007 per 1 SD higher ABCA1-CEC) in patients with low CRP. In bDMARD nonusers ABCA1-CEC associated with increased plaque progression (HR 2.65 per 1 SD higher ABCA1-CEC, p=0.001). We demonstrated that CLC and CEC strongly, and independently from known risk factors, correlate with coronary atherosclerosis and with the risk of cardiovascular events in patients with RA. Lipoprotein function evaluated as CLC and CEC, especially considered together with the other mentioned factors (inflammation, autoantibodies, PCSK9, drugs) may help classifying CVR in AR patients and applying optimal individual strategies for prevention and treatment.

Relevance to Conditioning Medicine: This study explores how CLC and HDL CEC could serve as novel biomarkers for CVR in RA patients. The study offers insights for personalized CVR management strategies in RA. This aligns with Conditioning Medicine's aim to advance tailored approaches for cardiovascular health through comprehensive risk assessment and understanding of underlying mechanisms.

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Hypertension exaggerates left ventricular hypertrophy in Fabry patients

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Left ventricular hypertrophy (LVH) and dysfunction are the main causes of death in patients with Fabry Disease (FD). Identification of clinical predictors of FD evolution is crucially important for the correct timing of therapeutic intervention. To evaluate the impact of hypertension (H) as a predictor of (LVH) progression in FD. We compared the FD database (319 patients) with patients from the URRAH study [>15000 patients] for H, indexed ventricular mass (LVMi) and calculated the odds ratio (OR) for LVH development. In a murine model of FD (tg-R301Q/KO mice) we chronically infused phenylephrine (PE,100 mg/Kg, 14-Days) by subcutaneous implantation of miniosmotic pump to increase blood pressure. Hypertension in FD compared to URRAH exploded the LVH-OR by 6.3 times. In H patients, LVMi is significantly higher in FD than URRAH patients (FD, no H:98.8±47; FD, H:148.8±67.5 g/m2 p<0.01; URRAH, no H:103.6±29.6; U, H:112.4±31.3 g/m2, p<0.01). The multivariate analysis for sex and age indicated that FD and hypertension interact as independent risk factors for LVH. PE-exposure induced a comparable increase in systolic and diastolic blood pressure in FD and control mice. However, in FD mice PE induced a larger increase of LVMi, alongside with higher Heart-body weight ratio, and higher ANP and MEF2 cardiac levels. In FD heart, PE caused energetic stress signaling activation suggesting the potential involvement of AMPK-FOX03-axis in the hypertrophic response of FD-heart under hemodynamic stress. Our data suggest that H is associated with a higher risk of LVH, representing a clinical predictor of a worsened evolution of FD cardiac phenotype. Data from the murine model confirm the exaggerated hypertrophic response to H of FD heart, probably due to energetic stress induced by the increased cardiac workload.

Relevance to Conditioning Medicine: This study investigates hypertension's impact on LVH progression in FD, a condition associated with cardiac dysfunction and mortality. Our findings suggest that FD hearts may display an exaggerated hypertrophic response to hemodynamic stress, contributing to Conditioning Medicine's efforts to understand and address cardiac stressors to enhance outcomes in cardiovascular comorbidities.

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Unveiling the biophysical mechanism of cardiac myocyte membrane potential modulation by a membrane-targeted photoswitch: an in silico investigation

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Optical stimulation is emerging as a promising alternative to conventional methods for both research and therapeutic purposes due to its advantages, such as reduced energy consumption, minimal invasiveness, and exceptional spatial and temporal precision. Recently we proposed Ziapin2, a newly synthesized light-sensitive azobenzene compound, as a tool to control cardiac cell excitability and contractility [1]. While Ziapin2 proved efficacy in precisely regulating the entire EC-coupling process in vitro in hiPSC-derived cardiomyocytes (CMs), the exact biophysical mechanism of action of the triggering process remains incompletely understood [2]. In this study, we aim to deepen our understanding of these mechanism by proposing an enhanced computational model of murine action potentials (APs) based on the Li et al. model from 2010 [3] that incorporates: i) the variation in membrane capacitance resulting from the trans-cis isomerization of the molecule in response to light stimulation; ii) stretch-activated ion channels (SACs) [4] potentially activated by membrane tension due to thickness variation induced by Ziapin2 isomerization.

The change in capacitance is modeled as:

$$C_m(dV/dt) = -I_{ion} - V(dC_m/dt)$$

where C_m is the specific cell membrane capacitance, V is the transmembrane voltage, t is time, and I_{ion} is the sum of all the ionic currents involved. While formulations for non-selective (K^+ and Na^+) and selective (K^+ and Ca^{2+}) SACs were implemented to model the contribution of these channels. Our numerical model accurately reproduces the alterations in cell capacitance and membrane potential induced by Ziapin2 photoisomerization. It elucidates the behavior observed experimentally in vitro in isolated mouse ventricular CMs, confirming the pivotal role of SACs in AP generation, particularly suggesting a significant involvement of selective Ca^{2+} channels. Overall, the availability of this in silico model, complementing experimental findings, fosters a comprehensive understanding of the phenomena underlying the Ziapin2-mediated photostimulation process, thereby opening interesting prospects for its application in cardiovascular research.

Relevance to Conditioning Medicine: We shed light on how light-sensitive compounds like Ziapin2 affect cardiac cell function. By uncovering the role of specific ion channels and membrane changes in Ziapin2's effects, our study offers insights for developing targeted interventions to address cardiac dysfunction. This aligns with Conditioning Medicine's goal of advancing tailored treatments for cardiovascular health.

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Electrophysiological in silico model of a 3 dpf zebrafish embryo

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Heart failure (HF) is a complex condition where the heart struggles to intake and/or eject sufficient blood, leading to high mortality rates, disability, and healthcare costs. Studying HF is difficult due to limited human cardiac tissue and diverse patient phenotypes. Hence, adaptable animal models like zebrafish are vital for research. Zebrafish, renowned for its genetic and embryonic traits, has become a key model for HF research during the last decades. Surprisingly, no numerical models for zebrafish HF have been developed to date. Therefore, this research aims to bridge this gap by creating an electrophysiological computational model of zebrafish, considering both the heart and the body. This model will deepen our understanding of the ionic mechanisms involved in cardiac conditions like arrhythmias. We constructed a finite element model for a 3-day post-fertilization (dpf) zebrafish embryo, including the whole body and the two chambers of the heart (i.e., atrium and ventricle). A detailed action potential model characterizes the electrical activity of various heart regions. Tissue conductivity is calibrated to reproduce the activation sequence observed experimentally. Additionally, by including the body, the extraction of both monopolar and bipolar ECG traces was enabled for comparison with existing experimental data [1][2]. To delve into ionic channels, we developed detailed action potential models for both the ventricle and atrium. Due to the lack of experimental data regarding plateau and background currents, as well as the pump and exchangers on zebrafish, the ventricular model was adapted from an existing human model from TenTusscher and Panfilov (2004) [3], reparametrizing steady-state and time constants to fit zebrafish physiology. The model was validated against experimental recordings under the same stimulation protocols (i.e., steady-state, S1S2, and dynamic protocols). Furthermore, we evaluated the model response to specific channel blockers (chromanol 239B, E-4031, and quinidine) using a pore block model, comparing results with literature data [4]. As experimental patch-clamp data for the atrium was unavailable in the literature, a preliminary model was derived from the ventricular model and validated using experimental AP and calcium transients. Integrating the electrophysiological finite element model with the detailed action potential models allowed us to assess the effects of specific channel blockers on APD and QT intervals in the ECG, facilitated by the incorporation of the body. This interdisciplinary approach, combining computational modeling with experimental validation, enhances our comprehension of zebrafish heart electrophysiology allowing us to investigate cardiac conditions and enhance drug testing methods. Moreover, the model aims to minimize animal usage in vivo experiments by refining experimental techniques.

Relevance to Conditioning Medicine: This study creates a computational model of zebrafish heart electrophysiology, integrating experimental data to provide insights into arrhythmias and aid drug testing. It aligns with Conditioning Medicine's aim to advance personalized cardiovascular treatments and reduce animal experiments.

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Extracellular vesicles a diagnostic tool to predict obstructive critical CAD in NSTE-ACS patients

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Acute coronary syndrome (ACS) represents a major cause of hospitalization, disability and death worldwide. After high-sensitivity troponin assay implementation, incidence of unstable angina (UA) drastically decreased, and patients were considered as a population with lower rates of death, major adverse cardiac events and obstructive coronary artery disease (CAD) compared with the non-ST elevation myocardial infarction (NSTEMI) group. However, UA patients showed higher probability of events of future myocardial infarction and coronary revascularizations compared with healthy subjects. Furthermore, according to the ESC clinical practice guidelines, a routine invasive strategy is recommended in both NSTEMI and UA patients during hospitalization. As a consequence, several coronary angiographies (CAGs) are performed in patients that, according to evidence, show considerable percentage of non-obstructive CAD. Therefore, the identification of valuable biomarkers to predict obstructive CAD in NSTE-ACS patients still represents an unmet clinical need. To address this issue, both free circulating microRNAs (miRs) and miRs enriched in extracellular vesicles (EVs) have been investigated as possible tools to stratify ACS patients. Based on our previous data, the aim of this study was to investigate the potential application of EVs recovered from NSTEMI and UA patients as biomarkers to predict obstructive CAD in these patients. Specifically, we analysed EV troponin content, their cell of origin, and their miR and protein content as predictors of obstructive critical CAD in NSTE-ACS patients. For this purpose, 140 patients with diagnosis of UA and NSTEMI were enrolled and underwent CAG. The study of the EV profiling showed that circulating EVs mainly originate from platelets and endothelial cells. Moreover, we found a significant correlation between EV CD62P surface marker, miR-130a-3p content and the absence of critical CAD. Finally, mass spectrometry of EVs showed a differential clusterization of EV protein cargo between patients with and without obstructive CAD. Notably, proteins associated with lipid transport/metabolism were found enriched in EVs from patients without obstructive critical CAD. Overall, our findings provide evidence for circulating EVs as predictive biomarkers of critical obstructive CAD in NSTE-ACS patients.

Relevance to Conditioning Medicine: The investigation of new predictive biomarkers of obstructive critical CAD in patients with UA or NSTEMI aligning with Conditioning Medicine's focus on understanding the mechanisms underlying these conditions for therapeutic purposes.

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ECG signals revisited with network science derived features

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Since the introduction of the electrocardiogram (ECG) by string galvanometer in 1901 by Prof. Einthoven, the interpretation of cardiac health has remained largely unchanged. Traditionally, experts analyze ECGs for diagnosis, but the increasing volume of data and advancements in computer-based methods necessitate new approaches for feature extraction. Network science is becoming a common language to describe complex systems. In network science, complex systems are represented as nodes connected with edges. Time series can be considered complex systems too and they can be translated into networks by using visibility graphs. It is possible to characterize these complex systems by calculating the graph properties and by using a cartography-based method. Cardiovascular signals, exhibiting strong pseudoperiodic behavior, present challenges in early detection of arrhythmias, a prevalent cardiovascular disorder. Early recognition as well a prediction of arrhythmias can potentially save many lives. We used the 2017 PhysioNet/CinC Challenge database, and we analysed 1,000 short ECG recordings (500 normal and 500 arrhythmic). One approach to ECG analysis via network science involves segmenting signals into chunks and transforming them into visibility graphs. The visibility condition states that it is possible to connect two time points if they are visible to each other, i.e. it is possible to connect the values of two time points without intersecting the values of the time points between them. Then, the multiple graphs from one ECG were overlapped, and the weights were normalised to obtain a weighted graph with weights between 0 and 1. We used an arbitrary threshold of 0.50 to cut the noisy edges. We obtained, in this way, a unique representative graph for the ECG of a subject. To extract the features that were used to classify an ECG, we performed community detection through Louvain algorithm, and we identified the roles of each node in the graph. The roles depend on the position of a node inside the community. We also calculated some properties of the graph spanning from the diameter to the density. The percentage of the number of nodes for each role, the total number of nodes, the average degree, the density, the diameter, and the average clustering were used as features for a random forest classifier. After optimising the hyperparameters of the machine learning classifier, we obtained an accuracy of 74% and an AUC of 0.81 on the test set (300 ECG recordings, 150 normal and 150 arrhythmic). This work can pave the path for revisiting the traditional ways of reading ECG based on the analysis of the typical ECG waves, such as the QRS complex, the P and the T waves. This work also presents an innovative way of using a cartography-based analysis of the network and of extracting new numerical features from an ECG signal. Finally, this work can help machines to better recognise the arrhythmic patterns absent in the normal signals.

Relevance to Conditioning Medicine: We introduce an innovative approach to ECG analysis, offering new insights into arrhythmia detection and classification. We provide a novel perspective on ECG signal interpretation, enhancing early detection and prediction of arrhythmias. This aligns with Conditioning Medicine's goal of advancing diagnostic methods and improving cardiovascular health outcomes through innovative technologies.

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Increasing research capacities in Kosovo, nanoparticles in environment and medical research – NanoKos

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The project NanoKos aims to significantly enhance innovation capacity and research activities in Kosovo, focusing on Environmental and Medical Research. NanoKos seeks to elevate research activities and empower Kosovo researchers to address societal challenges and contribute to global scientific advancement through strategic partnerships with leading European institutions. NanoKos aims to cultivate cooperation between EU institutions and the University of Prishtina (UP) and boost research activity. The project will provide: i) modern research equipment; ii) comprehensive training in research techniques for Kosovo researchers and involve students in research-based learning; iii) facilitate easier access to participate in conferences and events and iv) strengthen interaction between researchers and stakeholders in priority research areas. Additionally, NanoKos aims to enhance public engagement and understanding of environmental and medical research issues, fostering a research-oriented culture. NanoKos's strategies encompass a variety of activities designed to build robust research capacities. These activities include internships, academic and professional visits, acquisition of cutting-edge research equipment, and initiatives that blend pure and applied research. Partnerships with institutions such as King's College London (KCL), the University of Milan (UNIMI), and the University of Parma (UNIPR) play a critical role. At UNIPR, researchers will gain insights into the electrophysiological and inflammatory responses in in-vivo rats following the intratracheal instillation of Prishtina particulate matter (PM2.5). This collaboration will be instrumental in understanding the health impacts of environmental pollutants. At UNIMI researchers have performed characterization of circulating lipid profile, cholesterol, and triglycerides, of human and mouse experimental models of lipid-induced metabolic syndrome. Researchers have performed protein and gene expression analysis on tissues to further investigate the impact of environmental changes. By acquiring new research skills and knowledge, and establishing extensive research collaborations and networks, the project seeks to elevate the quality and scope of research conducted at UP. The project also intends to improve the research infrastructure and management capacities, making it a more competitive institution internationally. It aims to increase the international visibility and recognition of UP researchers in the EU, encourage evidence-based policy-making based on research findings, and develop research-oriented culture. By contributing to overall social well-being and sustainable development, NanoKos represents a strategic investment in Kosovo's research ecosystem, fostering innovation, collaboration, and excellence in Environmental and Medical Research.

Relevance to Conditioning Medicine: NanoKos represents a strategic investment in research ecosystem, fostering innovation, collaboration, and excellence in Environmental and Medical Research, which are themes of Conditioning Medicine.

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Exploring istaroxime unknown effects on pulmonary artery smooth muscle cells

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Smooth muscle cells (SMCs) demonstrate versatility, transitioning between contractile and proliferative states in response to vascular conditions. These changes are associated with alterations in the expression of Ca^{2+} handling proteins and intracellular Ca^{2+} levels. Istaroxime, able to inhibit Na^+/K^+ ATPase and stimulate SERCA2a, is considered a promising agent for acute heart failure treatment. Moreover, istaroxime inhibits prostate cancer cells migration associated to decreased Store Operated Calcium Entry (SOCE). This study investigates the effects of istaroxime on intracellular Ca^{2+} dynamics and proliferation of rat pulmonary artery (rPA) SMCs, aiming to uncover its potential role in vascular diseases beyond the cardiac ones. rPASMCs were characterized for α-smooth muscle actin (α-SMA) positivity using western blot and immunofluorescence analysis, ensuring the purity of the cell culture. Drug effects on intracellular Ca^{2+} dynamics were evaluated using Fluo4-AM for single-cell analysis and Fura2-AM for cell population analysis. Na^+/K^+ ATPase current (INaK) was assessed as K^+out activated current in isolated voltage-clamped rPASMCs (hp -40 mV). Finally, rPASMC proliferation was quantified using the CCK-8 kit. rPASMCs treated for 48 hours with istaroxime (1μM) showed decreased resting Ca^{2+} levels; opposite effects were observed with the selective SERCA blocker, CPA. Since the selective SERCA2a stimulator PST3093 (the main istaroxime metabolite) did not affect resting Ca^{2+} levels, a SERCA-independent effect of istaroxime was hypothesized. The drug significantly reduced SOCE, while ATP-induced SR Ca^{2+} release was unaffected by the drug; moreover, the potency of Na^+/K^+ ATPase inhibition was comparable to that observed in cardiac preparations. Finally, istaroxime reduced rPASMC proliferation. Overall, these results suggest that istaroxime modulates intracellular Ca^{2+} dynamics and proliferation of rPASMCs through a SERCA-independent mechanism, possibly through SOCE inhibition. Therefore, istaroxime might represent a new candidate for vascular diseases treatment.

Relevance to Conditioning Medicine: This study explores istaroxime's potential in treating vascular diseases by investigating its effects on PASMCs. By modulating intracellular calcium dynamics and inhibiting proliferation, istaroxime shows promise as a therapeutic candidate beyond its known cardiac applications. These findings support the pursuit of innovative treatments for vascular conditions, in line with Conditioning Medicine's aim of advancing cardiovascular health through novel therapeutic approaches.

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Cardiac microtissues capture RNA splicing events essential for heart maturation and identify isoform diversity in doxorubicin-induced cardiotoxicity

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The first human heartbeat occurs at 5-6 weeks of gestational age. Though the remarkable experience of hearing the sinus rhythm for the first time, it also states the heart is the first organ to develop. The expression of different gene isoforms needs to be coordinated in a timely manner during heart development and this is largely obtained by RNA Alternative Splicing (AS). However, a comprehensive insight into the cardiac-specific splicing program is still missing. Here we performed an AS analysis to identify isoforms essential for heart development. We identified cardiac-specific isoforms from those sharing expression with other tissues, especially skeletal muscle, and brain. Furthermore, we derived developmental trajectories, from early gestational ages to adulthood, for the most significant splicing events and with reference to RNA Binding Proteins (RBPs) expression. First, we leveraged these results to assess the developmental stage of in vitro models of the human heart. We developed 2-dimensional (2D) hiPSC-derived cardiomyocytes (CM) and 3-dimensional (3D) hiPSC-derived cardiac microtissues (MT) and compared their maturation stage based on the quantification of selected AS events. Analysis shows 2D-CMs roughly correspond to the human heart at 4-6 weeks of age and that 3D-MTs shift towards 10-12 weeks of age. Then, we used 2D-CMs and 3D-MTs to detect splicing alterations in doxorubicin-induced cardiotoxicity (DICT). Doxorubicin (doxo) is a chemotherapeutic drug largely used for its efficacy in killing tumours. However, DICT is a major side-effect causing morbidity and mortality among oncology patients, especially the young ones. 2D-CMs have been largely used to study DICT in vitro but their immaturity is a barrier to the recapitulation of post-natal heart disease and remodelling. Here, we used mature 3D-MTs and the cardiac splicing events we shortlisted to detect RNA isoform diversity in DICT. We found that cardiac gene isoforms display a substantial drift towards immature stages of heart development in 3D-MTs treated with doxo, at early phases of cardiotoxicity. Altogether, we identified a set of RNA splicing events tightly connected to the heart maturation and DICT pathology. Results could help identify cues we are still missing to advance maturation of in vitro models of the human heart. Moreover, they could also be useful to detect cancer patients' predisposition to DICT in the near future.

Relevance to Conditioning Medicine: We investigated RNA splicing patterns essential for heart development and their response to DICT. This study enhances our understanding of in vitro heart model maturation and offers insights into potential biomarkers for assessing susceptibility to DICT in cancer patients. Thus aligning with Conditioning Medicine's goal of advancing cardiovascular health through improved understanding of both responses to stress and therapeutic strategies.

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NLRP3 inflammasome inhibitors as pharmacological agents to counteract human endothelial dysfunction

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Endothelial inflammation, induced by oxidative stress and lipotoxicity, contributes to the development of endothelial dysfunction. Since ROS generation and lipotoxicity are associated with NLRP3 inflammasome activation, we aim to assess the effect of selective NLRP3 inflammasome inhibitors, INF195 and INF150, on endothelial cell dysfunction induced by oxidative stress and lipotoxicity. Human Coronary endothelial cells (HCAECs) were stimulated with 250-500 μM H_2O_2 or

100-250 μM palmitate and cell viability and angiogenesis in vitro were assessed. The addition of 250 μM and 500 μM H_2O_2 significantly impaired cell viability by 59 ± 7 and 29 ± 3.7, respectively. The NLRP3 inhibitors demonstrated concentration-dependent and significant prevention of cell death, with greater efficacy at the lower H_2O_2 concentration. Treatment of HCAEC with palmitate (250-100 μM) significantly impaired cell viability that was in part prevented by NLRP3 inflammasome inhibitors. Palmitate significantly impaired the ability of endothelial cells to form pseudocapillay structures, which were slightly attenuated by the NLRP3 inhibitors. These data suggest that NLRP3 inhibitors may in part prevent stress-induced endothelial dysfunction.

Relevance to Conditioning Medicine: This study investigates the efficacy of selective NLRP3 inflammasome inhibitors in preventing endothelial dysfunction induced by oxidative stress and lipotoxicity. Understanding their impact aligns with Conditioning Medicine's goal of developing strategies to alleviate cardiovascular stress and injury, potentially offering new therapeutic avenues for endothelial dysfunction.

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Elucidating the clinical phenotype of patients affected by genetic cardiomyopathy in a 3D beating heart-on-chip platform

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Cardiomyopathies (CMPs) are the leading cause of heart failure worldwide. CMPs represent a heterogeneous group of diseases (e.g., hypertrophic, dilated, arrhythmogenic and left ventricular non compaction) and they can manifest through a wide variety of symptoms. Recently, genetic testing has proven to be important to better classify both affected patients and individuals who are asymptomatic but at risk of developing CMPs. In this context, the combined use of human-induced Pluripotent Stem Cell-derived Cardiomyocytes (h-iPSC-CMs) and Organs-on-Chip (OoCs) represents a promising approach to generate relevant in vitro disease models recapitulating the key traits of CMPs, unlocking the possibility to study cell genotype-phenotype relationship and to develop patient-specific therapies. Here we present a functional patient-specific 3D tachycardia myocardial model developed within a beating Heart-on-Chip platform (uHeart) that is used for drug screening. The pathological model was developed by culturing h-iPSC-CMs differentiated from familial cases carrying a polymorphism of RyR2, both in heterozygosity and homozygosity. h-iPSC-CMs were combined with wild type human cardiac fibroblasts (h-CFs) in a 75%-25% ratio, embedded in fibrin hydrogel (125*10^6 cells/ml) and inoculated into the uHeart platforms. Microconstructs were cultured for up to 9 days and subjected to mechanical stimulation through uBeat® patented technology, which provides the 3D cultures with a physiological uniaxial cyclic strain (i.e., 10% stretching, 1 Hz). Upon achievement of functional microtissues synchronously and spontaneously beating, Field Potential (FP) of both models was acquired through a system of integrated electrodes (i.e., μECG) featured in uHeart. FP morphology was analysed, allowing to assess electrophysiological parameters (i.e., beating period-BP, spike amplitude-AMP, FP duration-FPD) and the onset of arrhythmic events. Caffeine and isoprenaline were selected to evaluate drug-induced alterations of electrophysiological cardiac signals. Both models resulted in spontaneously beating cardiac microtissues after 6-8 days of mechanical training, with an average BP of 1.4 ± 0.4 s for heterozygous and of 1.3 ± 0.5 s for homozygous microconstructs. Both models showed reduced BP and FPD when subjected to caffeine (5 mM) and isoprenaline (1μM). In accordance with preliminary clinical evidence, homozygous cells exhibited a higher tendency to develop arrhythmic events, as detected by online electrophysiological recording, that further increased upon treatment with both compounds.Our Heart-on-Chip platform enabled the generation of functional 3D in-vitro models of a genetic CMP, showing potentiality to provide relevant data to support elucidation of clinical phenotype.

Relevance to Conditioning Medicine: We present functional 3D tachycardia myocardial models using h-iPSC-CMs and OoCs. By mimicking CMPs, these models aid in studying genotype-phenotype relationships and testing personalized therapies. Through electrophysiological monitoring and drug screening, they offer insights into CMP pathophysiology, advancing tailored treatment strategies for cardiovascular health; thus fitting with aim and scope of Conditioning Medicine.

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The microtubules plus-end tracking proteins CLIP-170 mediates nuclear shape in Emery-Dreifuss muscular dystrophy

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Mutations in the lamin A/C (LMNA) gene are associated with an autosomal dominant inherited form of Emery-Dreifuss muscular dystrophy (EDMD), which is characterized by slowly progressive muscle weakness and wasting, as well as dilated cardiomyopathy (DCM). The pathological mechanisms underlying this disorder remain unclear, which has limited the development of therapeutic strategies. Therefore, we set out to unravel the molecular and cellular mechanisms underlying EDMD in $Lmna^{p.H222P/H222P}$ mice, a model of this disease that also recapitulates key features of LMNA-cardiomyopathy. We recently demonstrated that a reduced acetylation of microtubules, a post-translational modification, was responsible for altered microtubule organization in EDMD. Here, we report that muscle fibres isolated from $Lmna^{p.H222P/H222P}$ mice also exhibited an altered distribution of tyrosinated α-tubulin. Given the established role of microtubules in regulating nuclear shape, this study aims to elucidate the mechanisms by which abnormal microtubule organization and their interaction with microtubule-associated proteins (MAPs) contribute to nuclear elongation, a cellular phenotype of EDMD. CLIP-170 is a MAP that binds to the plus-end of microtubules to protect them from depolymerization. We found that CLIP-170 displays a punctiform localization at the poles of elongated nuclei in muscles fibres isolated from $Lmna^{p.H222P/H222P}$ mice, while it is localized around the nuclei in the wild-type animals. CLIP-170's activities are contingent upon conformational changes. A folded conformation of CLIP-170 (phosphorylated form) dissociates from microtubule plus ends. Conversely, CLIP-170

in the open extended conformation (unphosphorylated form) binds microtubules with greater affinity. We investigated the effect of a neurosteroid that activates CLIP-170 by altering its conformation. We discovered that this drug restores the nuclear shape in striated muscles of EDMD mice by removing CLIP-170 from the poles of elongated nuclei. These findings suggest that CLIP-170 plays a critical role in modulating nuclear shape in EDMD.

Relevance to Conditioning Medicine: This study investigates the molecular mechanisms of EDMD, linked to LMNA gene mutations. It focuses on the role of CLIP-170, a microtubule-associated protein, in regulating nuclear shape abnormalities in EDMD. The findings suggest that modulating CLIP-170 activity with a neurosteroid restores normal nuclear shape in EDMD mice, offering potential therapeutic insights for this condition; thus fitting with aim and scope of Conditioning Medicine.

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Insulin-dependent GLUT4 membrane translocation in hiPSC-derived cardiomyocytes

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) are a cell model now widely used to investigate patho-physiological features of cardiac tissue [1]. Given the invaluable contribution hiPSC-CM could make for studies on cardio-metabolic disorders by defining a postnatal metabolic phenotype, our work herein focused on monitoring the insulin response in CM derived from the hiPSC line UKBi015-B. In particular, after hiPSC-CM differentiation according to standard protocols we add a purification phase in lactate-containing medium to trigger an effective metabolic maturation of differentiated cells [2]. Western blot analysis on total cell lysates obtained from hiPSC-CM showed the relative expression of two glucose transporters, GLUT4 and GLUT1, whose expression switch marks out the transition from the fetal to the post-natal phenotype [3]. We therefore focused on the characterization of the insulin response in our model of lactate-purified hiPSC-CM. Western Blots on total lysates showed increased phosphorylation of both AKT and AS160 following insulin treatment. Furthermore, in order to monitor GLUT4 dynamics in response to metabolic regulators, Western blot analysis of plasma membrane fractions, rather than total lysates, revealed insulin-induced plasma membrane translocation of GLUT4, as occurs in adult CM [4]. Thus, these findings suggest that hiPSC-derived CM exhibit an insulin response reminiscent to that of a post natal CM regarding intracellular signaling and GLUT4 translocation to the plasma membrane, thus representing a suitable cellular model in the cardio-metabolic research and personalized medicine field.

Relevance to Conditioning Medicine: We studied the insulin response in hiPSC-CM, providing insights into their suitability as a cellular model for studying cardio-metabolic disorders. By demonstrating insulin-induced signaling and GLUT4 translocation similar to adult cardiomyocytes, this research enhances our understanding of cellular metabolism and offers potential applications in personalized medicine within the cardio-metabolic research field; thus aligning with aim and scope of Conditioning Medicine.

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Sinoatrial node heterogeneity and fibroblasts increase atrial driving capability in a two-dimensional human computational model

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Cardiac pacemaking remains an unsolved matter under many points of view. Extensive experimental and computational research has been performed to describe sinoatrial physiology across different scales, from the molecular to the clinical level. Nevertheless, how the heartbeat arises inside the sinoatrial node and propagates to the working myocardium is, at present, not fully understood. This work aims at providing quantitative information about this fascinating phenomenon, especially regarding the contribution of cellular heterogeneity and fibroblasts to sinoatrial node automaticity and atrial driving. This is achieved by developing a bi-dimensional computational model of human right atrial tissue including the sinoatrial node. State-of-the-art knowledge of anatomical and physiological aspects was adopted during the design of the baseline tissue model. The novelty of this study is the presence of cellular heterogeneity and fibroblasts inside the sinoatrial node to investigate how they tune the robustness of stimulus formation and conduction under different conditions (baseline, ionic current blocks, autonomic modulation, external high frequency pacing). The simulations show that both heterogeneity and fibroblasts significantly increase the safety factor for conduction by more than 10% in almost all the conditions tested and shorten the sinus node recovery time after overdrive suppression up to 60%. In the human model, especially in challenging conditions, fibroblasts help the heterogeneous myocytes to synchronize their rate (e.g., -82% in σCL under 25 nM acetylcholine administration) and to capture the atrium (with 25% L-type calcium current block). However, anatomical and gap junctional coupling aspects remain the most important model parameters to allow an effective atrial excitation. In conclusion, despite the limitations of the model, this work suggests a quantitative explanation to the astonishing overall heterogeneity shown by the sinoatrial node.

Relevance to Conditioning Medicine: The study of cellular crosstalk (myocytes-fibroblasts communications) inside the human heart to understand its role in physiological and pathological cardiac pacemaking using computational models. In particular, it is investigated how the sinoatrial node reacts to and recovers from overdrive pacing due to arrhythmia.

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Development of analytical methods for detecting air contaminants and microplastics in water

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Environmental pollution is one of the most pressing risks and challenges facing the world today. Humans encounter various pollutants through ingestion, inhalation, and skin contact, leading to numerous health issues such as ischemic heart disease, stroke, chronic obstructive pulmonary disease, leukemia, and cancer. The World Health Organization (WHO) reports that air pollution causes millions of deaths globally each year, accounting for nearly a quarter of all fatalities [1–3]. In the Republic of Kosovo, there is a significant lack of scientific data on the levels of organic contaminants in both indoor and outdoor air, as well as on microplastics in water. Moreover, the association between air and plastic pollution and cardiovascular issues underscores the urgent need for a national program to promote and enforce air quality and water standards, with a specific focus on microplastics [4–6]. To address this knowledge gap, our research aims to develop analytical methods based on various ISO standards to identify and quantify organic contaminants across different environmental media, with a particular emphasis on air pollutants and microplastics in water together with contaminants that they may have sequestered from the environment. We will employ instrumental techniques such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), infrared spectroscopy (IR), and other analytical methods. Once developed, these methods will be applied to monitor outdoor and indoor air quality, as well as microplastics in water, in Kosovo. This will enhance the understanding of pollution levels, help identify the sources of these pollutants, and facilitate the development of strategies to reduce or eliminate them from the environment. Ultimately, our goal is to contribute to public health improvements and environmental protection by informing policy and raising awareness about pollution issues in Kosovo.

Relevance to Conditioning Medicine: We address the critical issue of environmental pollution and its potential impact on cardiovascular health. The findings can inform policy and raise awareness to promote public health improvements and environmental protection, aligning with Conditioning Medicine's goal of advancing cardiovascular health through comprehensive approaches to address environmental factors.

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Investigations of cellular and molecular mechanisms of LMNA-cardiomyopathy: insight from iPSC-derived cardiac models.

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Cardiomyopathy caused by mutation in the LMNA gene (LMNA-CMP), encoding the nuclear lamina proteins Lamin A and C, is a form of inherited cardiomyopathy belonging to a group of rare disorders, the laminopathies. These lead to a wide range of phenotypes affecting various tissues, including the myocardium. The primary cardiac manifestation is dilated cardiomyopathy, often accompanied by a high incidence of conduction abnormalities and fatal arrhythmias. These symptoms can vary significantly among individuals, and there is no clear correlation between specific genotypes and phenotypes. Several hypotheses have been proposed to account for this clinical heterogeneity, including epigenetic regulation. We recently demonstrated an effect of a LMNA mutation (p.K219T) on the transcription of SCN5A, encoding the cardiac ion channel (Nav1.5), which has a key function in cardiac conduction and action potential generation. These findings represent the first report in CMs in which epigenetic regulation driven by defective Lamin A/C is a potential cause of cardiac disease; however, the mechanisms by which LMNA mutations alter the electrical/mechanical properties of human heart cells remain under-assessed. Our goal is to further deepen the contribution of Lamin A/C-mediated epigenetic processes to LMNA-CMP phenotypes. To this, we used cardiomyocytes differentiated from patient-specific induced pluripotent stem cells (iPSC-CMs) carrying LMNA mutations and the respective isogenic and healthy controls.Results from RNA sequencing revealed over 1400 genes differentially expressed between LMNA- and control CMs, mostly involved in cardiac conduction, contraction and metabolism. Biochemical studies in LMNA-CMs confirmed a significant reduction in the expression of contractility genes such as TNNCI and TRIM63 and found increased binding of Lamin A/C to their promoters. These features were associated to a reduced contractility, delayed Ca^{2+} transients, and increased beating frequency in response to beta-adrenergic stimulation in LMNA-CMs. In conclusion, these results are in support of an epigenetic effect of Lamin A/C mutations also on CMs contractile function through a direct regulation of specific target genes. Additional studies are now ongoing directed to determine the distinct roles of Lamin A/C in CMs subtypes (atrial, nodal and ventricular), and will be crucial to clarify on the heterogeneous clinical phenotype associated to the disease, underlying more specific genotype/phenotype correlations.

Relevance to Conditioning Medicine: We investigated the impact of LMNA gene mutations on cardiomyocyte contractility and electrical properties. By utilizing iPSC-CMs, we shed light on the underlying mechanisms of laminopathies. Understanding how these mutations affect gene expression

and cellular function could lead to improved diagnosis and treatment strategies; thus aligning with Conditioning Medicine's objective of advancing knowledge in cardiovascular health to develop tailored therapeutic interventions.

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Simulated hypovolemia by lower-body negative pressure affects venous pulse wave velocity in upper limbs

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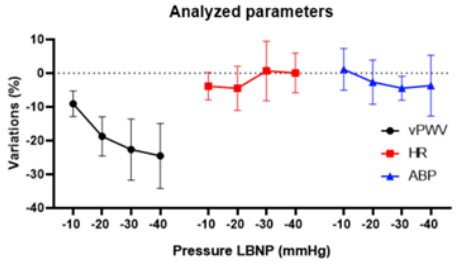
The propagation velocity of a pulse wave in blood vessels depends on the stiffness of the vessel wall, which in turn depends on transmural pressure and the extent of distension of the vessel wall, which is related to vascular filling. Recent studies suggest that the pulse wave velocity in peripheral veins (vPWV) could be used as a convenient non- invasive indicator of volemic status [1, 2]. Aim of this study is to investigate the dependence of vPWV on increasing simulated hypovolemic stimuli as produced by lower-body negative pressure (LBNP). Six young healthy subjects (age 28.3 ± 13.2, 3 males, 3 females) were subjected to a randomized sequence of LBNP stimuli (from -10 to -40 mmHg, for 1-2 min) while monitoring vPWV (in the left arm), arterial blood pressure (ABP) and heart rate (HR). vPWV was measured by a custom-made device generating a pulse pressure by a pneumatic compression of the wrist and detecting the propagating pulse wave in the basilic vein by Doppler ultrasound [3]. A continuous monitoring of vPWV could be performed for the whole duration of the experimental session (above 30 min). A progressive decrease of vPWV was on averaged detected with increasing LBNP: 0.24 ± 0.13, 0.50 ± 0.17, 0.60 ± 0.27, 0.66 ± 0.33 m/s at -10, -20, -30, -40 mmHg, respectively ($p<0.05$, one-wayANOVA) while no significant changes in arterial blood pressure and HR were detected. These preliminary results indicate that changes in vPWV are a sensitive marker of volume status changes, and that this methodology is suitable for long-term recordings and is thus a promising tool to monitor the effectiveness of fluid therapies in patients.



Relevance to Conditioning Medicine: This study explores the potential of using vPWV as a non-invasive indicator of volume status. Data suggest that vPWV decreases with increasing LBNP, indicating its sensitivity to changes in volume status. This method offers promise for monitoring fluid therapies in patients, aligning with Conditioning Medicine's focus on advancing diagnostic tools and treatment strategies for cardiovascular health.

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