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Cor et Vasa

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Czech Cardiovascular Research and Innovation Days 2025 & 11th European Section Meeting of the International Academy of Cardiovascular Sciences,

Prague, November 2–4, 2025

Cor et Vasa

S PŘÍLOHOU

Kardio

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Cor et Vasa

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Cor et Vasa, the journal of the Czech Society of Cardiology and Czech Society for Cardiovascular Surgery, publishes 6 times a year and covers all aspects cardiology, angiology, cardiovascular surgery, cardiovascular imaging, pediatric cardiology, hypertension, cardiovascular prevention and some aspects of interventional radiology.

It features editorial, original articles, review articles, as well as short communications from clinical and experimental cardiology. Beginning 2012, *Cor et Vasa* has also been publishing summaries (5 000 words) of the European Society of Cardiology guidelines, developed by leading Czech experts in the field. Beginning 2025, *Cor et Vasa* **does not accept case reports**. Should you be interested in publishing a case report, please submit your contribution to Cor et Vasa Case Reports, a sister journal of Cor et Vasa, to be accessed at https://www.kardio-cz.cz/coretvasa-case-reports.

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IS IKUR MORE THAN AN ATRIAL CURRENT? EFFECT OF SELECTIVE IKUR INHIBITION ON CANINE AND HUMAN CARDIAC VENTRICULAR AND PURKINJE FIBERS

Abdelmagid AAE[1], Topal L[1], Mohammed ASA[1], Mohacsi G[1], Paskuj B[1], Naveed M[2], Demeter-Haludka V[1], Deri S[1], Bitay G[1], Kohajda Z[1], Nagy N[1], Virag L[1], Baczko I[1,3], Jost N[1,3], Varro A[1,3]

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Introduction: Several pharmaceutical companies developed selective inhibitors of IKur/Kv1.5 current to treat atrial fibrillation. These efforts were motivated by the consensus that IKur was expressed and functioned primarily in the atrial tissue, with little to no functional expression in ventricular myocytes. Consequently, it was anticipated that these drugs would circumvent the proarrhythmic side effects commonly linked to the currently available therapies. Recent findings, however, have begun to challenge this view, pointing to a potential role for IKur in the ventricular myocardium and renewing debate over its presence and physiological relevance. Therefore, our study aimed to investigate the possible existence and function of IKur within cardiac ventricular muscle.
Methods: Immunocytochemistry was utilized to detect the potential expression of Kv1.5 channels in isolated canine ventricular myocytes. Action potentials were recorded using standard conventional microelectrode techniques from both the canine right and left ventricular papillary muscle and Purkinje fibers. Patch-clamp measurements were conducted to measure IKur in isolated canine ventricular and Purkinje myocytes. Selective inhibitors (XEND0103 and 4-aminopyridine (4-AP)) for IKur were applied to determine IKur.
Results: Immunocytochemistry studies confirmed the abundant Kv1.5 expression in ventricular myocytes, challenging the prior belief of its atrial exclusivity, and in canine cardiac Purkinje cells. Action potential recordings demonstrated a significant prolongation of APD90 in both canine and human ventricular (280.7 ± 7.5 ms vs. 292.0 ± 7.0 ms, n = 10, p ≤0.05) and Purkinje fibers (230 ± 9 ms vs. 258 ± 9 ms, n = 8, p ≤0.005) after treatment with 1 µmol XEN-D0103. Patch-clamp experiments showed that XEND103 and 4-AP sensitive current corresponding to IKur are present in cardiac ventricular and Purkinje myocytes. In those dog left ventricular muscle preparations, where repolarization reserve had been previously weakened, IKur inhibition by XEND103 and 4-AP elicited an augmented response, suggesting its role in repolarization reserve in the ventricle.
Conclusion: Contrary to the prevailing belief, our experimental findings suggest that IKur is indeed expressed in ventricular muscle and its inhibition prolongs cardiac ventricular repolarization. Also, IKur can be considered as an important contributor to the repolarization reserve.

CLINICAL OUTCOMES IN PATIENTS WITH CARDIOGENIC SHOCK TREATED WITH WEARABLE CARDIOVERTER DEFIBRILLATOR: DATA FROM A MULTICENTER INTERNATIONAL REGISTRY

Abumayyaleh M[1,*], Hoffmann LM[1,*], Schupp T[1], Koepsel K[2], Erath JW[3], Kuntz T[4], Klein N[4], Kovacs B[5], Duru F[5], Saguner AM[5,6], Blockhaus C[7,8], Shin DI[7,8], Kreimer F[9], Gotzmann M[9], Lapp H[10], Beiert T[10], Aweimer A[2], Mugge A[2,11], Weiß C[12], El-Battrawy I[2,11], Akin I[1]

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Background: Cardiogenic shock (CS) remains a complex condition associated with a high mortality rate. However, the use of wearable cardioverter defibrillators (WCDs) in this high-risk population remains poorly investigated.
Methods: We retrospectively analyzed 730 patients treated with WCDs across multiple centers, dividing them into two cohorts: 134 patients with CS and 596 without CS. Baseline characteristics, WCD usage data, and clinical outcomes were compared between the two groups. A multivariable analysis was performed to identify predictors of mortality.
Results: Patients with CS were more likely to have a history of coronary artery disease (CAD) (59% vs. 47.8%, p = 0.020), ischemic cardiomyopathy (ICM) (46.3% vs. 33.1%, p = 0.004), and heart failure (HF) (47.8% vs. 32.4%, p = 0.001). WCD wear duration was longer in CS patients (median 71.0 [39.5–99.0] vs. 64.0 [40.0–91.3] days, p = 0.003), with a higher proportion of CS patients wearing the device for more than 90 days (37.6% vs. 28.6%, p = 0.042). No significant differences were observed in ventricular arrhythmic events or shocks between the groups. CS patients had a higher rate of device implantation at follow-up (54.5% vs. 34.5%, p < 0.001), but mortality (9.7% vs. 6.4%, p = 0.172) and rehospitalization rates (46.6% vs. 44.3%, p = 0.692) did not differ significantly. Predictors of mortality included age (odds ratio [OR] 1.052, 95% confidence interval [CI] 1.021–1.083, p = 0.001) and prior ICM (OR 2.387, 95% CI 1.023–5.569, p = 0.044).
Conclusion: Patients with CS treated with WCDs exhibited higher rates of comorbidities and device implantation but had similar mortality and rehospitalization outcomes compared to those without CS. Prolonged WCD use was observed in patients with CS. Prior ICM was identified as a negative predictor of long-term mortality.

AGE VARIATION IN PATIENTS WITH TROPONIN LEVEL ELEVATION WITHOUT OBSTRUCTIVE CULPRIT LESION OR SUSPECTED MYOCARDIAL INFARCTION WITH NON-OBSTRUCTIVE CORONARY ARTERIES – LONG-TERM DATA COVERING OVER DECADE

Abumayyaleh M[1,2], Schlettert C[3], Materzok D[3], Mugge A[3,4], Hamdani N[5,6,7], Akin I[1], Aweimer A[3], El-Battrawy I[4,5,8]

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Background: Troponin level elevation without an obstructive culprit lesion is caused by heterogenous entities. The effect of aging on this condition has been poorly investigated.
Methods: After screening 24,775 patients between 2010 and 2021, this study included a total of 373 patients with elevated troponin levels without an obstructive culprit lesion or suspected myocardial infarction with non-obstruc-

tive coronary arteries (MINOCAs) categorized into four age groups containing 78 patients (<51 years), 72 patients (51–60 years), 81 patients (61–70 years), and 142 patients (>70 years). This study analyzed the baseline characteristics, the in-hospital complications, in-hospital mortality, and the long-term outcomes.
Results: The older patients exhibited a higher rate of major adverse cardiovascular in-hospital events than those of the other age groups (15.4% in the <51-year-old group vs. 36.1% in the 51–60-year-old group vs. 33.3% in the 61–70-year-old group vs. 47.2% in the >70-year-old group; p <0.001). However, the rate of non-sustained ventricular tachycardia (nsVT) was higher in the 51–60-year-old patients than those of the other age groups (5.6% in the 51–60-year-old group vs. 1.3% in the 61–70-year-old group vs. 0.7% in the >70-year-old group; p = 0.027). At the 11-year follow-up, cardiovascular mortality was higher among the older patients compared to that of the younger patients (3.9% in the 61–70-year-old group vs. 4.2% in the >70-year-old group, p = 0.042), while non-cardiovascular mortality was comparable between the age groups.
Conclusions: The older patients with troponin level elevation without an obstructive culprit lesion experienced a higher incidence of major adverse cardiovascular events during hospitalization compared to that of the younger groups. Additionally, higher cardiovascular mortality rates were revealed in the older patients at a long-term follow-up.

DYNAMIC PERFORMANCE ASSESSMENT OF ATHLETES AND ITS RELATION TO CARDIOVASCULAR RISK

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Introduction: Elite sports place extreme demands on athletes' cardiovascular systems. Long-term exposure to high physical exertion can lead to adaptations that, on one hand, enable peak performance but, on the other hand, may pose potential health risks for athletes in the long run. The aim of our study was to assess the dynamics of performance parameters over five years and analyze their relationship to established norms and potential cardiovascular risk.
Methodology: The study included 3,033 athletes aged 9–25 years, monitored between 2019 and 2023. The following parameters were assessed: age, weight, height, BMI, resting and maximum heart rate (HR_{rest}, HR_{max}), resting and maximum systolic and diastolic blood pressure (SBP_{rest}, DBP_{rest}, SBP_{max}, DBP_{max}), maximum power output (W), maximum power output relative to body weight (W/kg), and maximal oxygen consumption (VO_{2max}). To evaluate performance, norms for W/kg were established, and athletes were divided into two groups: "below norm" and "within norm".
Results: During the five-year observation period, we noted dynamic changes in the percentage of athletes meeting the established W/kg norms. These changes were evident both within specific age categories and across the overall cohort. In 2019, 64.5% of athletes met the norm, while in 2023, this percentage was 64.9%. The largest year-over-year increase was observed in the category of 15-year-old athletes, where the proportion meeting the norm rose from 82.9% in 2019 to 91.8% in 2023. Conversely, the largest year-over-year decline was recorded in the category of 11-year-old athletes, where the proportion decreased from 60.2% in 2019 to 42.0% in 2023.
Conclusion: Our study demonstrated that the performance of elite hockey players changes dynamically over time and that these changes are specific to individual age categories. Long-term monitoring of performance parameters and their relationship to cardiovascular risk is essential for optimizing the training process and ensuring the long-term health of athletes.

ANALYSIS OF THE QUANTITY OF PROTEIN PRODUCTS OF THE TITIN GENE IN PATIENTS WITH DILATED CARDIOMYOPATHY

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Objective: Protein-terminating (truncating, ttv) mutations in the titin gene (*TTN*) represent a frequent cause of inherited heart failure (HF), but its molecular mechanism is not yet fully understood. The aim of our study was to quantify the protein products of the *TTN* alleles in the left ventricular (LV) myocardium.
Sample and methodology: 44 samples of explanted LV during Tx (29/44 dilated cardiomyopathy [DCM], 15/44 controls, 38 men, mean age 44 ± 14 years) were collected. Based on previous genetic analysis from blood there were 19/29 DCM-TTNtv+ and 10/29 DCM-TTNtv– with mutations in *LMNA* (3×), *TNNT2* (2×), *FLNC, BAG3, SGCD, MYH7*, *PLN*. Control myocardium was obtained from organ donors with normal LV function. Using Western blotting on a two-phase polyacrylamide gel, we determined the relative amounts of titin isoforms, N2BA, N2B, and Cronos, normalized to total or sarcomeric protein (MHC).
Results: The total amount of all titin isoforms was highest in controls and lowest in DCM-TTNtv–. The expression of N2BA and N2B was identical in all sample groups, whereas the expression of T2 and Cronos was higher in controls and lower in DCM, regardless of genotype. The N2BA/

N2B ratio, responsible for diastolic stiffness, was the same among the DCM groups.
Conclusions: The expression of wt protein products of the *TTN* gene is lower in HF patients than in non-HF controls. However, the amount of wt titin does not differ between DCM-TTNtv+ and DCM-TTNtv–, indicating a possible toxic effect of TTNtv+ products as suggested before.

IMMUNOBIOLOGY OF UNSTABLE PLAQUES IN CAROTID ARTERY AND NOVEL TREATMENT STRATEGIES

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Arterial occlusive disease is the principal cause of ischemic syndromes affecting every circulatory bed of the human body. The central feature of atherosclerosis is a chronic inflammatory response in the vessel wall involving many immune cells including macrophages, dendritic cells, and T-lymphocytes and local effects of cytokines and growth factors. The products of activated immune cells are critical in the development, progression and instability of atherosclerotic plaques. Patients with carotid artery disease are broadly classified as symptomatic or asymptomatic patients. Symptomatic patients due to chronic inflammation and rupture of vulnerable atherosclerotic plaque and thrombus formation in carotid arteries show active symptoms including transient ischemic attack, stroke, aphasia and other motor defects. Prevention of atherosclerotic plaque formation and stabilization of a vulnerable plaque is of therapeutic significance to decrease the number of acute events. We have reported an imbalance between the inflammation and reparative process tending to plaque instability. The findings supported the critical role of TLR-4 and TREM-1 in the underlying pathophysiology of chronic inflammation, atherosclerotic plaque formation, and plaque instability. We examined the effect of the selective inhibitors of TREM-1 (LR-12) and TLR4 (TAK242) in microswine model of carotid artery atherosclerosis. Hypercholesterolemic Yucatan microswine were treated with a control vehicle and TREM-1 or TLR4 inhibitor at the time of intimal injury and were sacrificed after 5–6 months. Optical coherence tomography (OCT) showed plaques with thick fibrous cap and occluded arteries in the controls. Radiologic evaluation of carotid arteries revealed decreased neointimal hyperplasia and plaque formation with the treatment with the TREM-1 inhibitor compared to scrambled peptide group. There was no significant effect of TAK242, when dissolved in 30% ethanol. However, TAK242 dissolved in clinically relevant vehicle significantly decreased the plaque size and enhanced plaque stabilization. Histomorphologically, there was neointimal hyperplasia with significantly increased inflammation and elastin degradation in the controls compared to the inhibitor groups on H&E and Movat-Pentachrome staining. The gene and protein expression for the markers of plaque vulnerability including MMP-7, IL-6, IL-12/23, and CD36 were significantly decreased with the treatment with TREM-inhibitor and TLR4 inhibitor (dissolved in clinically relevant vehicle) compared to individual vehicle control. The findings of this study support the therapeutic efficacy of inhibiting either TLR-4 or TREM-1 signaling alone or in combination in attenuating atherosclerotic plaque vulnerability, reduce plaque burden, and thus prevent the occurrence of transient ischemic attack and stroke.

Supported by the research grant R01 HL144125 by the National Institutes of Health, USA.

HIF-1α SIGNALING MEDIATES CARDIOPROTECTION THROUGH METABOLIC AND MITOCHONDRIAL REMODELING IN CHRONIC HYPOXIA

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Background: Hypoxia-inducible factor 1-alpha (HIF-1α) is a key transcriptional regulator of cellular response to hypoxia. We previously demonstrated that the cardioprotective effect of adaptation to chronic hypoxia (CH) against ischemia/reperfusion (I/R) injury relies on the presence of HIF-1α. To further elucidate the molecular mechanisms underlying this protection, we investigated the influence of HIF-1α on cardiac metabolism, mitochondrial function and dynamics, and oxidative stress.
Methods: Male wild-type (WT) mice and mice with partial Hif1a deficiency (Hif1a+/–) were exposed to CH for 4 weeks, while controls remained under normoxia. Isolated perfused hearts were then subjected to I/R to determine infarct size. We performed RNA-sequencing of isolated cardiomyocytes and mitochondrial respiration and substrate preference were analyzed in left ventricular tissue. Mitochondrial dynamics and mitophagy were examined by electron microscopy, western blotting, and Mito-Keima assay. Oxidative stress and the role of mitochondrial permeability transition pore (mPTP) opening were also evaluated. A multi-omics approach was conducted to assess HIF-1α-dependent changes.

Results: Adaptation to CH enhanced ischemic tolerance in WT mice compared with the normoxic controls and chronically hypoxic Hif1a+/– mice. This cardioprotection was mediated through HIF-1α-activated mitophagy. Bulk mRNA sequencing revealed substantial CH-induced transcriptional reprogramming in cardiomyocytes, with a prominent focus on mitochondrial pathways. In WT mice, CH reduced mitochondrial respiration and altered mitochondrial ultrastructure. CH also upregulated antioxidant enzymes, decreased oxidative stress markers, and reduced oxidative stress, as evidenced by lower reactive oxygen species (ROS) production during fatty acid oxidation. Furthermore, CH influenced mPTP opening, implicating this mechanism in cardioprotection. We also identified HIF-1α-dependent metabolic and proteomic rewiring under CH conditions.
Conclusion: Our findings demonstrate that HIF-1α plays a crucial role in cardiomyocyte survival during acute I/R injury by promoting selective autophagy and coordinating additional protective metabolic and mitochondrial adaptations.

TOPICAL APPLICATION OF IMMORTELLE ESSENTIAL OIL-ENRICHED OINTMENT MITIGATES OXIDATIVE STRESS AND INFLAMMATION IN WOUND TISSUE

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Background and aim: *Helichrysum italicum* (immortelle) is a Mediterranean plant from the *Asteraceae* family, traditionally used for treating wounds and burns due to its anti-inflammatory, antioxidant, and antibacterial properties. However, scientific evidence supporting these effects is still limited. This study aimed to formulate an ointment containing immortelle essential oil and evaluate its effects on wound healing in streptozotocin-induced diabetic rats, with a focus on tissue redox status and inflammatory mediators.
Materials and methods: Thirty-two *Wistar albino* rats with diabetes mellitus were used. After confirmation of hyperglycemia, full-thickness excision wounds (2 × 2 cm) were created on the dorsal area. Rats were randomly divided into four groups (n = 8): Group I (untreated), Group II (ointment base), Group III (0.5% immortelle ointment), and Group IV (1% silver sulfadiazine). Treatments were applied topically once daily for three weeks. After three weeks, the rats were sacrificed and the wound tissues were collected. Biochemical analysis of wound tissue included measurement of pro-oxidant marker, thiobarbituric acid reactive substance (TBARS), parameters of antioxidative defense including reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), and inflammatory cytokines (IL-6, TNF-α, IL-10) using ELISA.
Results: The immortelle group showed the highest antioxidant enzyme activity (CAT and SOD) and significantly reduced oxidative stress in wound tissue. Levels of proinflammatory cytokines TNF-α and IL-6 were markedly lower compared to the untreated and vehicle control groups. Notably, IL-10, an anti-inflammatory marker, was significantly elevated in the immortelle-treated group, indicating a favorable shift in the local inflammatory response.
Conclusion: These findings support the dual antioxidative and anti-inflammatory effect of immortelle essential oil in diabetic wound healing.

ELECTROPHYSIOLOGICAL AND ANTIARRHYTHMIC EFFECTS OF A NEW ANALOGUE OF MEXILETINE

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The antiarrhythmic and cardiac electrophysiological effects of a new mexilatine analogue, SZV-2649, were studied in mammalian cell lines, in rat and dog cardiac preparations and in vivo in rats and dogs. SZV-2649 exerted antiarrhythmic effects in a coronary artery occlusion/reperfusion-induced arrhythmia model in rats and in acetylcholine- and burst stimulation-induced atrial fibrillation in Beagle dogs. SZV-2649 inhibited the hERG and GIRK currents in HEK cells (with IC_{50} of 342 and 529 nM, respectively). In canine ventricular myocytes, SZV-2649 reduced the densities of I_{Kr}, I_{to} and I_{NaL} and I_{CaL} currents. The compound elicited Class IB type V_{max} reducing and Class III type action potential duration prolonging effects in dog right ventricular muscle. In canine atrial muscle, SZV-2629 moderately prolonged the action potential duration and this effect was greatly augmented in preparations pretreated with 1 μM carbachol. In conclusion, SZV-2649 exhibited antiarrhythmic effects based on its multiple ion channel blocking properties. Since its chemical structure shows major differences compared to that of amiodarone, it is expected that SZV-2649 may exhibit fewer adverse effects and may be a promising molecule for further development.

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THE ROLE OF UROLITHIN A IN PREVENTION OF ISOPRENALINE-INDUCED HEART FAILURE IN RATS

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Background: Urolithin A (UA) is a natural metabolite derived from ellagitannins, produced by gut microbiota.[1–3] It is known that UA improves muscle strength, exercise performance, and biomarkers of mitochondrial health.[4] This study aimed to evaluate the cardioprotective effects of urolithin A in isoprenaline-induced HF in rats.
Methods: Isoprenaline (85 mg/kg, s.c.) administered on two consecutive days was used to induce HF in male Wistar rats. The rats were treated with UA (5 mg/kg/day i.p.) for 14 days after being exposed to isoprenaline. The experimental animals were divided into 4 groups: Ctrl (control, received saline on days 1 and 2 + vehicle for 14 consecutive days), Isp (isoprenaline on days 1 and 2 + vehicle for 14 days), UrlA (saline on days 1 and 2 + UrlA for 14 days) and Isp+UrlA (isoprenaline on days 1 and 2 + UrlA for 14 days). In this study, biochemical parameters, oxidative stress, and hemodynamic parameters were evaluated.
Results: Biochemical analysis revealed that UA significantly reduced plasma levels of oxidative stress markers including thiobarbituric acid reactive substances (TBARS), nitrites (NO_2–), and hydrogen peroxide (H_2O_2). Antioxidant defence was enhanced, as shown by significant increases in levels of superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT). Additionally, UA treatment led to a marked reduction in myocardial matrix metalloproteinases MMP-2 and MMP-9, thereby mitigating heart remodelling and preventing HF. Echocardiography showed improved ejection fraction, suggesting that UA has promising potential in the prevention of HF.
Conclusion: These findings indicate the promising role of UA as therapeutic agent for preventing HF through its cardioprotective mechanisms.

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IRON DEFICIENCY IN PATIENTS AFTER HEART TRANSPLANTATION

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Introduction: Iron deficiency is a common comorbidity in patients with advanced heart failure, significantly affecting their quality of life, hospitalisations and functional capacity. Heart transplantation induces substantial changes in iron metabolism, influenced by inflammatory responses and repeated blood transfusions perioperatively. This study aimed to compare iron metabolism parameters in patients before and after heart transplantation.
Patients and methods: The study included 23 patients who underwent heart transplantation (HTx) by 2023 and 2024, compared their iron parameters before and after HTx. Analyzed parameters were serum iron, transferrin saturation, ferritin and hemoglobin and statistical analysis was performed.

Results: Iron deficiency was present in 70% of patients with advanced heart failure and in 52% after HTx. Before HTx, ferritin levels were (median – IQR) 122.9 (63.6–224.2), after HTx was ferritin statisticly significantly higher, 298.3 (126.9–659.7), p <0.01, some of the patients had extremely elevated ferritin levels. In pre-transplant patients, transferrin saturation was 0.15 (0.11–0.19) and after HTx it was also significantly higher, 0.22 (0.15–0.30), p <0.01. In serum iron there was not significant difference in patients before and after HTx. After HTx, higher prevalence of anaemia was present, with haemoglobin levels of 126.0 (118.0–148.0) before HTx and 119 (106.0–123.5) in post-transplantation patients, p <0.01. These findings suggest that inflammation and repeated transfusions play a critical role in altering iron metabolism in transplanted patients.
Conclusion: Heart transplantation is associated with significant changes in iron metabolism parameters. In particular, the increase in ferritin levels probably reflects the inflammatory state and the effects of repeated blood transfusions during and after surgery. The high ferritin and persistent anemia observed in patients after transplantation may have clinical implications requiring further monitoring and targeted interventions.

CARDIOPROTECTIVE POTENTIAL OF FLAVONOID QUERCETIN IN AGED DIABETIC FATTY RATS

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Despite extensive research in the field of cardioprotection, there are still no effective drugs on the market for the prevention/treatment of myocardial ischemia-reperfusion (I/R) injury. Therefore, there is a great need to search for new cardioprotective compounds. Quercetin (QCT), a natural polyphenol enriched in human food, is a promising compound that has several beneficial effects on the cardiovascular system, including the prevention of cardiac I/R injury. The cardioprotective potential of QCT has been extensively documented in healthy young animals, but limited data are available on the effects of QCT on the heart in comorbidities and in aging subjects.
The aim of this study was to investigate the potential cardioprotective effects of QCT in aging subjects suffering from the metabolic comorbidity – type 2 diabetes (T2DM). An animal model of T2DM, Zucker diabetic fatty rats (ZDF) of two different ages, 6-month-old and 1-year-old, was used for this purpose. QCT at a dose of 20 mg/kg/day was administered orally for 6 weeks. The effects of QCT on cardiac morphology and function *in vivo* were monitored by echocardiography. After the end of treatment, hearts were isolated and subjected to I/R ex vivo (30-min global ischemia/2-h reperfusion). Recovery of cardiac function and infarct size were assessed as physiological outcomes of the experiments. Molecular signaling pathways involved in the effect of QCT were assessed by Western blotting.
The results showed that QCT has cardioprotective effects on the diabetic heart *in vivo* by preventing diastolic dysfunction and fibrosis, but is ineffective in preventing I/R injury in aged T2DM rats. In conclusion, QCT may be potentially cardioprotective in elderly diabetic rats, but aging and the presence of diabetes may reduce or even abolish its anti-ischemic effects.

ESTABLISHMENT OF A REGISTRY FOR PATIENTS WITH SPONTANEOUS CORONARY ARTERY DISSECTION (SCAD): RATIONALE, DESIGN, AND PRELIMINARY INSIGHTS

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Background: Spontaneous coronary artery dissection (SCAD) is an important but under-recognized cause of acute coronary syndromes (ACS), particularly in young to middle-aged women without traditional cardiovascular risk factors. Recent observational studies suggest that SCAD accounts for up to 1–4% of all ACS cases and up to 35% in women under 50 presenting with myocardial infarction. Despite growing awareness, data on incidence, management strategies, and long-term outcomes remain limited, highlighting the need for systematic data collection.
Methods: We propose a prospective single-center registry enrolling patients with angiographically or intravascular imaging-confirmed SCAD. Baseline demographic, clinical, angiographic, and management data will be collected.
This registry aims to characterize the clinical presentation, diagnostic pathways, treatment approaches, and long-term prognosis of SCAD patients. By creating a database, we seek to improve understanding of SCAD's natural history, identify potential risk factors, and facilitate the development of evidence-based management guidelines.
Preliminary results: We have included 13 patients in the single-center registry so far. Pilot data from our cohorts suggest a predominance of female patients (84.6%) with an average age of 47.5 ± 4.9 years. 53.8% patients were initially presented as STEMI and overal 76.9% patients had single vesell disease. The most frequently affected

artery was the LAD, in 53.8% of cases and total of 46.2% of patients were treated with PCI. Other findings included an average ejection fraction of 50% and an average high-sensitive troponin I value of 3028.5 ± 2947.5 ng/l. SCAD recurrence rates have been reported worldwide to be between 10–30% after 5 years, in our center the recurrence was 23% within 3 years, highlighting the need for long-term follow-up.
Conclusion: A dedicated SCAD patient registry will provide comprehensive epidemiological and clinical insights, support collaborative research, and ultimately contribute to improved diagnostic and therapeutic strategies for this unique patient population.

A TRANSLATIONAL TWO-HIT HFPEF MOUSE MODEL REFLECTING DIASTOLIC DYSFUNCTION, PULMONARY HYPERTENSION, AND ENDOTHELIAL IMPAIRMENT

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Introduction: Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome linked to metabolic and hypertensive comorbidities, characterized by diastolic dysfunction despite preserved ejection fraction. The absence of a reliable animal model replicating the complex HFpEF phenotype hampers mechanistic and translational research. To address this, we characterized a 2-hit mouse model combining metabolic (obesity/T2DM) and hemodynamic stress (chronic d-aldosterone infusion).
Methods:10-week-old Leprdb/db and C57/BL6J WT mice (n = 5–10, both sexes) were used. Leprdb/db mice (obese, DM=1st hit) received a 4-week d-aldosterone infusion (0.3 µg/h minipump = 2nd hit), referred to as HFpEF-mice. Cardiac function was assessed by echocardiography, vascular reactivity by wire myography. Lung hemodynamics were measured using a 1F Millar catheter to assess the right ventricular systolic pressure.
Results: LVEF was elevated in HFpEF mice (81% vs. 66% in controls, $p \leq 0.0001$), while diastolic function was impaired, consistent with a reduced heart rate (463 ± 47 vs. 542 ± 60 bpm in controls, $p \leq 0.0087$). Mitral E/A (1.46 vs. 1.24, $p \leq 0.016$), and E/e' (44.20 vs. 24.01, $p \leq 0.001$) were elevated. LA area was enlarged (3.70 vs. 2.43 mm², $p \leq 0.012$). Heart weight-to-tibia length ratio was significantly increased in HFpEF-mice (8.23 vs. 5.97 mg/mm, $p < 0.0001$). Strain analysis showed reduced reverse peak strain rate (–10.64 vs. –23.92% 1/s, $p \leq 0.019$) and lower global longitudinal strain (–16.15 vs. –22.68%, $p \leq 0.056$) in HFpEF mice vs. control. Right heart catheterization revealed elevated RVSP (58.90 vs. 19.10 mmHg, $p \leq 0.0320$) and mPAP (37.93 vs. 13.65 mmHg, $p \leq 0.0199$) in HFpEF-mice, indicating increased RV afterload and pulmonary hypertension, supported by a higher lung wet-to-dry ratio (6.623 vs. 5.012 mg, $p \leq 0.05$).
Endothelial dysfunction in 2-hit mice was indicated by impaired endothelium-dependent relaxation in renal artery (38.99% vs. 64.89%, $p \leq 0.0371$) and thoracic aorta (32.88% vs. 76.72%, $p < 0.0001$), along with enhanced vasoconstriction (renalis: 120.50% vs. 109.61%, $p = 0.5566$; aorta: 157.20% vs. 109.52%, $p \leq 0.0126$) compared to controls.
Conclusion: This 2-hit model recapitulates key features: supranormal EF with diastolic dysfunction, LV hypertrophy, LA remodeling, pulmonary congestion, and endothelial dysfunction.

FUNCTIONAL ANALYSIS IN THREE PROBANDS SUFFERING FROM "TRUE" IDIOPATHIC VENTRICULAR FIBRILLATION

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Variants in cardiac ion channel genes, common causes of various inherited arrhythmias, can be detected in some patients suffering from "true" idiopathic ventricular fibrillation (VF). These variants are frequently classified as variants of uncertain significance, thus, their causality is uncertain. Functional analysis is then the only way to consider their possible pathogenic character.
We currently perform functional analysis on three probands suffering from idiopathic VF, the first one carrying two KCNH2 (hERG) variants (A228V and S1021Qfs*98; IKr channel), the second one a single RYR2 variant (Y4734C; RYR2 channel), and the third one a single SCN5A variant (M135V; INa channel). KCNH2 and SCN5A variants are tested using the whole-cell patch clamp technique on human ion channels with and without the specific variant transiently expressed in a cell line. The analysis revealed that S1021Qfs*98-KCNH2 is a pathogenic, dominant-neg-

ative variant with substantially decreased expression, resulting in a decrease of the repolarizing current through channels co-expressing A228V and S1021Qfs*98 variants by ~65%. The pilot data in M135V channels show a decreased depolarizing current and several disturbances of gating, including rightward shifts of both activation and inactivation voltage dependences and faster recovery from inactivation. In the Y4734C-RYR2 variant, patient-specific hiPSC-derived cardiomyocytes of the proband, his sister with the same variant and diagnosis of catecholaminergic polymorphic ventricular tachycardia, and his healthy nephew were prepared and are being tested using the multielectrode array technique and simultaneous recording of Ca^{2+} transient and contraction (IonOptix). A significantly higher short-term variability of the cycle length in both control and proarrhythmic conditions (hypokalemia, fever, β-stimulation) was observed in both carriers of Y4734C-RYR2, the proband and his sister, compared to healthy cardiomyocytes of the proband's nephew; the highest irregularity of the rhythm was identified in the proband. Analysis under application of sex-hormones is followed to explain the differences between Y4734C-RYR2 cardiomyocytes of the male proband and his sister.

Functional analysis enables detailed characterization of ion channel dysfunction. In the case of idiopathic VF, it may provide essential data for understanding arrhythmogenesis and for clinical management of both probands and previously asymptomatic carriers of the same variant.

ASSOCIATION OF GALECTIN-3 GENE POLYMORPHISM WITH MYOCARDIAL INFARCTION RISK

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Introduction: Galectin-3, encoded by the *LGALS3* gene, is involved in immune regulation, inflammation, and metabolic disorders such as obesity, type 2 diabetes, and atherosclerosis. Due to its broad involvement in various diseases, galectin-3 is considered as a potential biomarker and therapeutic target. The rs4644 polymorphism in *LGALS3* may influence susceptibility to myocardial infarction (MI) through regulation of galectin-3 expression, which plays a key role in post-infarction inflammation and myocardial remodelling. This study aimed to investigate the association between the rs4644 polymorphism and MI risk in patients from the Republic of Srpska.

Material and methods: The case-control study included 93 MI patients not taking lipid-lowering drugs and 93 age- and sex-matched healthy controls. The blood samples were obtained from the Cardiology Clinic of the University Clinical Centre of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina. *LGALS3* rs4644 genotyping was performed using qPCR.

Results: The results have shown that the patients with the AA genotype had significantly higher total cholesterol and triglyceride levels compared to C allele carriers (p = 0.040), indicating a potential association between this genotype and dyslipidaemia in MI. Additionally, MI patients carrying the AA genotype showed significantly higher BMI values compared to controls (p = 0.048). Assessment of risk for MI showed that carriers of the A allele (CA+AA) had a statistically increased risk compared to individuals with the CC genotype (p = 0.047).

Conclusion: This study suggests that the *LGALS3* rs4644 polymorphism could be associated with increased MI risk and adverse lipid profiles. A larger sample size would provide more reliable results.

GREEN TEA POLYPHENOLS INHIBIT METABOLIC SYNDROME-ASSOCIATED CARDIOMYOPATHY BY MODIFYING ENDOGENOUS REACTIVE SULFUR SPECIES

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Objectives: Obesity and metabolic syndrome are often associated with abnormal cardiac function and structure even in the absence of comorbidities such as diabetes or hypertension, the condition referred to as obesity cardiomyopathy. Recently, we have demonstrated that green tea polyphenols have beneficial effects on endothelial cells and perivascular adipose tissue by converting endogenous hydrogen sulfide (H2S) to polysulfides (H2Sn). In the present study we examined the effect of green tea extract (GTE) on the experimental model of MS-associated cardiomyopathy.

Methods: MS was induced in adult male rats by feeding high fructose diet (20% fructose in the drinking water) for 2 months. Cardiac function was examined by measuring ±dP/dt and the expression of genes/proteins associated with cardiac hypertrophy and remodeling was assessed by RT-qPCR and Western blotting or ELISA methods. H2S

and H2Sn production in myocardium was measured by electrochemical sensors and specific fluorescent probes.
Results: Fructose feeding induced small increase in body weight gain and adiposity. Blood pressure and fasting glucose remained normal in fructose-fed rats, however, fasting insulin and triglyceride levels were markedly elevated. Fructose had no effect on +dP/dt but reduced –dP/dt suggesting diastolic dysfunction. In addition, fructose increased heart weight/body weight ratio as well as the expression of F4/80 (the marker of macrophage infiltration), fibrosis score, 3-nitrotyrosine (3-NT, the marker of oxidative/nitrosative stress), TGF-beta, ANP, and BNP. Fructose feeding increased Akt and mTOR phosphorylation and reduced phospholamban phosphorylation in left ventricular myocardium. Green tea extract (GTE) administered for 2 weeks at a dose of 10 mg/kg improved diastolic function, reduced cardiac hypertrophy and fibrosis, reduced the expression of F4/80, 3-NT, TGF-beta, ANP and BNP, decreased Akt and mTOR phosphorylation and increased phospholamban phosphorylation. These effects were mimicked by H2Sn donor, Na2S4, but not by H2S donor, Na2S. In addition, GTE increased H2Sn and reduced H2S level in the heart. GTE had only minor effect on insulin and triglyceride levels.
Conclusions: Green tea polyphenols improve myocardial diastolic function and reduce myocardial hypertrophy and remodeling associated with the metabolic syndrome independently of systemic metabolic profile. The effect of green tea is associated with converting H2S to polysulfides.

CHARACTERISTICS AND OUTCOMES OF ACUTE CORONARY SYNDROME IN YOUNG PATIENTS: A SINGLE-CENTRE ANALYSIS

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Introduction: The incidence of acute coronary syndrome (ACS) in patients under 50 years of age is increasing. Data of early-life cardiovascular morbidity and its impact on mid-term mortality in ACS is however limited. The purpose of this study is to better understand clinical characteristics and outcome of young ACS patients and to examine treatment strategies, survival rates and causes of death in this subgroup.
Methods: We analysed single-centre data of all ACS patients over a five-year period (from October 2018 to October 2023). Patients ≤50 years old were selected and further analysed. Their characteristics – including initial presentation, a set of 13 risk factors, treatment approach, and discharge therapy – were assessed. We further analysed national health institute mortality records, categorized into cardiovascular and non-cardiovascular causes.
Results: Of 3,199 patients admitted with ACS, 331 (10.3%) were ≤50 years old, with a male-to-female ratio of 80% (n = 264) to 20% (n = 67), respectively. The mean age was 44.8 ± 4.5 years, with the youngest patient being 22 years old. The mean body weight was 91.3 ± 18.1 kg. The mean left ventricular ejection fraction (LVEF) was 49.4 ± 10%. Each patient had an average of 2.7 ±1.6 risk factors, with smoking being the most common (83%, n = 275). Among clinical presentations, 54% (n = 180) had STEMI, 27% (n = 90) NSTEMI, 12% (n = 40) unstable angina, and 6% (n = 20) myocardial infarction with non-obstructive coronary arteries (MINOCA). Percutaneous coronary intervention (PCI) was performed in 83.4% (n = 276) of cases, while 10.8% (n = 36) underwent coronary artery bypass grafting (CABG). At discharge, 91% (n = 301) received dual antiplatelet therapy (DAPT), while 9% (n = 30) were prescribed anticoagulation therapy. The overall survival rate over a mean follow-up period of 3.5 ± 1.4 years was 95.8%. Cardiovascular causes accounted for 71% (n = 10) of deaths, while non-cardiovascular causes accounted for 29% (n = 4) of deaths. Mean time intervals from an ACS to death were 130 ± 314 days for cardiovascular causes and 737 ± 535 days for non-cardiovascular causes (p = 0.303).
Conclusion: ACS patients under 50 years old have an excellent mid-term survival. However, cardiovascular mortality accounts for the majority of deaths and occurs earlier compared to non-cardiovascular. These findings highlight the need for tailored secondary prevention strategies in young ACS patients to mitigate early cardiovascular mortality.

INTEGRATIVE *IN VIVO* AND *IN SILICO* ANALYSIS OF EXERCISE-INDUCED CARDIOPROTECTION IN HYPERTENSIVE AND NORMOTENSIVE RATS

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Introduction: Exercise training is known to exert cardioprotective effects; however, the molecular mechanisms underlying these benefits remain poorly defined. This study aimed to explore the influence of exercise on cardiac function and neurotrophin-related molecular signaling pathways in spontaneously hypertensive rats (SHR) and Wistar rats. We further integrated molecular docking to characterize structural interactions involved in exercise-induced adaptation.

Methodology: Male SHR and Wistar rats were stratified into control and voluntary wheel running (VWR) groups with free access to running wheels for 5 weeks. Echocardiographic assessments (cardiac output, heart rate, fractional shortening, relative wall thickness) were conducted pre- and post-training. BDNF, TrkB, VGF, CREB, Bicc1, Glut1, and gC1qR expression were measured via RT-PCR; protein expression was evaluated by Western blot in left ventricular tissue. 3D models of BDNF and TrkB (Rattus norvegicus) were generated using AlphaFold, and structures retrieved from the Protein Data Bank (e.g., BDNF PDB: P23363, TrKB: Q63604). These were used for preliminary docking to explore structural interactions. Group-specific docking, based on experimental data, was planned to characterize potential differences in binding behavior.
Results: Post-training, Wistar rats significantly increased cardiac output and fractional shortening (p <0.05), indicating improved function, whereas SHR showed no benefit and increased wall thickness. Heart rate remained unchanged in both strains. TrkB, VGF, CREB, and Glut1 expression were significantly higher in running Wistar vs. SHR (p <0.015), with moderate to large effect sizes. Bicc1 increased significantly in running vs. control groups (p <0.0001); gC1qR remained unchanged. Docking confirmed stable BDNF–TrkB interactions with salt bridges (e.g., LYS25/26–ASP349), hydrogen bonds (e.g., TYR86), and hydrophobic contacts (e.g., TRP19), with minimal inter-chain distance of 8.7 Å.
Conclusions: VWR significantly enhanced cardiac performance and upregulated neurotrophin-related factors TrkB, VGF, and CREB in Wistar but not SHR rats. Significant changes in Glut1 and Bicc1 suggest additional metabolic or post-transcriptional mechanisms. These results indicate a strain-dependent limitation in exercise-induced cardioprotection, potentially due to impaired neurotrophin signaling in hypertension. Docking supports the key role of BDNF–TrkB interaction in mediating these adaptations.

IDENTIFICATION OF DIAGNOSTIC AND PROGNOSTIC BIOMARKERS IN HEART FAILURE PATIENTS UNDERGOING LEVOSIMENDAM TREATMENT

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Introduction: Heart failure (HF) is a progressive clinical syndrome associated with high morbidity and mortality, particularly in patients with advanced stages of the disease. Levosimendan, a calcium sensitizer and inodilator, enhances myocardial contractility without significantly increasing oxygen consumption. The identification and monitoring of circulating biomarkers such as neprilysin (NEP) and growth differentiation factor 15 (GDF-15) may provide valuable insights into treatment response and support both diagnostic and prognostic stratification.
Objectives: This study aimed to evaluate changes in selected circulating biomarkers in patients with advanced heart failure undergoing levosimendan infusion and to assess their potential utility as diagnostic and prognostic indicators of therapeutic response.
Materials and methods: Seventy-five patients with New York Heart Association (NYHA) class III–IV heart failure who received levosimendan therapy were enrolled. Serum samples were collected immediately before and after the levosimendan infusion. Concentrations of NEP and GDF-15 were quantified using commercially available ELISA kits.
Results: Levosimendan infusion induced measurable changes in circulating biomarker levels. GDF-15 levels showed a statistically significant decrease (p = 0.0094) from pre-treatment baseline (median = 1,794 ng/L [min–max: 162.3–7,805 ng/L]; n = 75) to post-treatment measurements (median = 1,633 ng/L [min–max: 269.0–5,271 ng/L]; n = 75), suggesting a biologically meaningful response to the intervention. Similarly, NEP concentrations showed a decreasing trend between pre- and post-infusion values (median = 1,527 ng/L [min–max: 277.6–21,542 ng/L]; n = 75 vs. median = 1,218 ng/L [min–max: 176.4–32,523 ng/L]; n = 75; p <0.0001). However, a subset of patients exhibited paradoxical increases in one or both biomarkers post-treatment, which may reflect a poor biological response to levosimendan and could be associated with suboptimal clinical outcomes.
Conclusion: Levosimendan therapy is associated with a significant reduction in circulating levels of NEP and GDF-15 in most patients with advanced heart failure, indicating a favorable impact on markers of cardiac stress and remodeling. The observed biomarker elevation in a minority of cases warrants further investigation to elucidate the underlying mechanisms and determine its prognostic implications.

INVESTIGATING THE ARRHYTHMOGENIC EFFECT OF ACTION POTENTIAL ALTERNANS IN HUMAN HEART FAILURE

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Background: Heart failure (HF) is a complex, progressive disease characterized by structural and electrical remodelling of the heart, significantly increasing the likelihood of ventricular fibrillation (VF). However, before VF develops, a potentially reversible process, known as alternans, may occur. Action potential (AP) alternans refers to the beat-to-beat oscillation of AP duration (long-short), leading to repolarization inhomogeneity.
Purpose: Since no specific pharmacological intervention exists to prevent alternans, our study investigated the effect of the selective Na^+/Ca^{2+}-exchanger (NCX) inhibitor ORM-10962 on alternans formation in both HF and healthy human hearts.
Methods: Tissue samples from HF and healthy hearts were used to conduct our experiments. HF samples were provided by the Városmajor Heart and Vascular Center, while healthy tissues were obtained from hearts of general human donors. APs were recorded using the conventional microelectrode technique on endocardial preparations.
Results: In HF samples, AP duration (APD) alternans were significantly larger than those observed in healthy hearts. In several cases, substantial amplitude alternans were detected in left ventricular HF hearts, primarily at higher pacing frequencies, further augmenting the APD alternans and eventually progressing to a 2:1 conduction block. Preparations presenting with amplitude alternans also showed slower AP amplitude restitution characteristics. Treatment with 1 μM ORM-10962 shortened the APD in HF samples, reduced the magnitude of APD alternans, and shifted the onset of amplitude alternans and conduction block to higher frequencies.
Conclusions: Electrical remodeling associated with heart failure significantly increases APD alternans, which appears to worsen with patient age, likely due to the progressive downregulation of SERCA. Specifically, failing hearts can develop amplitude alternans at faster heart rates, which markedly enhances the magnitude of APD alternans. The underlying mechanism may involve impaired recovery of the sodium current. Selective NCX inhibition may attenuate alternans in the human failing heart at slower heart rates, possibly due to APD shortening and its effects on calcium handling.

ANTIOXIDANT AND VASODILATATORY PROPERTIES OF MEDICINAL PLANT *GERANIUM ROBERTIANUM* L.

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Background: Natural products with antioxidative and vasoactive properties are of increasing interest as therapeutic adjuvants or compounds with potential for drug development. *Geranium robertianum* L., a medicinal plant used in traditional medicine, is thought to confer beneficial effects on vascular function through antioxidant and antihypertensive mechanisms. This study aimed to determine the antioxidant potential and to evaluate the vascular activity of its methanolic extract.
Material and methods: Antioxidant activity was quantified using a panel of established *in vitro* assays – DPPH, ABTS, FRAP, and CUPRAC – to assess radical scavenging and redox potential. In order to examine the mechanisms of *Geranium robertianum* L. – induced relaxation, functional tests were applied to the organ bath system. Isolated rat thoracic aortic rings were precontracted with phenylephrine (10^{-5} M), followed by application of the extract. To investigate the role of the endothelium in the vasorelaxant response, the L-NAME (10^{-4} M) was administered.
Results: *Geranium robertianum* L. extract exhibited remarkably strong antioxidant activity across multiple assays, including DPPH (IC_{50} = 10.17 μg/mL), ABTS (IC_{50} = 2.55 μg/mL), CUPRAC (5.74 mmol TE/g), and FRAP (6.07 mmol TE/g). In vascular reactivity tests, the extract (1mg/ml) caused up to 40% relaxation of phenylephrine-precontracted vessels, demonstrating a noteworthy vasodilatory effect. This effect was significantly reduced by L-NAME (10^{-4} M), suggesting an endothelium-dependent mechanism likely mediated by nitric oxide.
Conclusion: These preliminary findings suggest that *Geranium robertianum* L. has promising cardiovascular potential. Further studies are needed to identify active ingredients and to elucidate molecular mechanisms related to vasodilatatory activity.

VALORIZATION OF WINE INDUSTRY BYPRODUCTS: UNLOCKING ANTIOXIDANT POTENTIAL FOR DIABETES-ASSOCIATED APPLICATION

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Background and aim: Chronic diabetic wounds are characterized by sustained oxidative stress and inflammation, both of which impair the normal healing cascade. This study evaluates the redox-regulating potential of a novel, plant-derived hydrogel formulated with grape skin extract (GSE) as a polyphenol-rich byproduct of the winemaking process. Typically discarded after vinification, grape skins represent a renewable and underutilized source of bioactive compounds.
Materials and methods: In a diabetic rat excision wound model, male *Wistar* rats were allocated into four groups: untreated control (NC), silver sulfadiazine-treated (PC), hydrogel without extract (HG), and hydrogel containing GSE (HG+GSE). After 15 consecutive days of topical application, wound tissue was analyzed for oxidative stress biomarkers such as thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH).
Results: The HG+GSE group exhibited a marked reduction in TBARS, suggesting lower lipid peroxidation. Simultaneously, antioxidants including SOD, CAT, and GSH were significantly elevated compared to all other groups. These improvements in redox balance are attributed to the sustained local release of potent polyphenols such as epicatechin, naringin, and caffeic acid determined in the highest concentrations in the GSE.
Conclusion: Our results highlight the hydrogel's potential as a biocompatible antioxidant therapy and demonstrate the value of repurposing grape pomace into a sustainable solution for diabetic wound management.

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HEART FAILURE WITH PRESERVED EJECTION FRACTION: THE NEW FRONTIER

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Heart failure with preserved ejection fraction (HFpEF) is a common, lethal, disabling, and expensive condition. Its prevalence has reached epidemic proportions (~3 million in the US and ~32 million people worldwide; ~1/2 of all cases of HF), and will continue to rise as obesity and diabetes increase and the population ages. The prognosis of HFpEF is poor, and mortality is at least as high as, if not greater than, that of patients with heart failure with reduced ejection fraction (HFrEF). Patients with HFpEF have an annual mortality of ~15%; in those hospitalized with HFpEF, the 5-year mortality is 50–75%, similar to lung cancer. In contrast to HFrEF, there has been little progress in the treatment of HFpEF in the past 40 years. Current therapies (diuretics and SGLT2 inhibitors) have limited efficacy. Thus, HFpEF constitutes a major (and growing) public health problem worldwide, a leading cause of morbidity and mortality, and an increasing burden on healthcare systems; it will soon become one of the greatest challenges in healthcare. Among the various subtypes of syndromes within the spectrum of human HFpEF (e.g., HFpEF induced by hypertension, renal disease, aging), the most prevalent is HFpEF driven by the metabolic syndrome (MetS), which accounts for 60-70% of all cases and is associated with obesity and hyperglycemia/insulin resistance. A major obstacle to progress in both the pathophysiology and therapy of MetS-induced HFpEF has been the lack of translationally relevant animal models, due to the difficulty in recapitulating the systemic, multiorgan dysfunction and comorbid-laden phenotype of patients with this syndrome. Most existing models of MetS-induced HFpEF use rodents, which are less relevant to the human syndrome and not suitable for testing device- based therapies. We have developed a new large animal model of MetS-induced HFpEF that fully recapitulates the key elements of the clinical syndrome, including impaired exercise capacity, and can be used for preclinical studies of new therapies. This is the only porcine model of HFpEF published heretofore that exhibits all of the following components: a full MetS, a full metabolic characterization, liver fibrosis and dysfunction, and reduced exercise tolerance. This porcine model is unique in that it recapitulates the entire constellation of major comorbidities and multiorgan abnormalities and the hemodynamic, clinical, and metabolic features of MetS-driven human HFpEF: obesity, arterial hypertension, hyperlipidemia, glucose intolerance, insulin resistance, liver fibrosis, liver dysfunction, pulmonary hypertension, increased LV filling pressures, concentric LV hypertrophy, LV diastolic dysfunction, preserved LV systolic function, and impaired exercise capacity. Because of its high clinical relevance, this model is well-suited for exploring the pathophysiology of MetS-driven HFpEF and the efficacy of new therapies.

DIABETES AND PREDIABETES IN CORONARY HEART DISEASE PATIENTS IN EUROASPIRE STUDIES

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Objective: In repeated European study of patients with stable coronary heart disease (CHD), we analysed diagnostics and treatment of impaired glucose metabolism in relation to other cardiovascular (CV) risk factors.
Design and method: In 6 consecutive surveys of the EUROASPIRE project, starting by 1994, we investigated men and women >18 ≤80 years old, after acute myocardial infarction and/or myocardial revascularization 6–24 months since index event. We retrieved case history data, measured body parameters, blood pressure, heart rate, and other, took blood samples for laboratory analysis, administered the questionnaires. Specificaly, in patients without known diabetes, we performed 2 hours oral glucose tolerance test.
Results: In the Czech study populations 400 patients per survey have been investigated (Prague and Pilsen center), in the last survey EUROASPIRE VI we analysed 200 patients in Prague center (Pilsen center abstained). In this last survey, only 26% of patients have normal glucose metabolism, prevalence of diabetes increased from 23,4 % in 1995 to 30% in this last survey (6% diagnosed by us, mainly due to 2h OGTT), we found impaired fasting glucose in 23%, impaired glucose tolerance in 21%. Prevalence of metabolic syndrome (namely obesity or severe obesity) was 85% in diabetics, severity of AMI (according to hs troponin levels, extent of coronary lesions, and prevalence of impaired left ventricular function according to TEE) was higher in diabetics than in nondiabetics. Lifestyle CV risk factors control (obesity and low physical activity, except smoking) in diabetics was mostly worse than in nondiabetics. On the other hand, blood pressure was well controlled in 75%, and lipid metabolism in 80% – better than in nondiabetics.
Conclusion: Prevalence of diabetes and prediabetes, is in patients with stable IHD very high. These patients have a higher risk profile and more advanced coronary impairment. 2h OGTT improves the diagnosis of impaired glucose metabolism and should be performed in pts post AMI.

THE CRITICAL ROLES OF HEALTHY GUT MICROBIOMES, ANTIOXIDANT DIETS, AND LIFESTYLE MODIFICATIONS IN PREVENTING CARDIOVASCULAR DISEASES

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The global rise in cardiovascular diseases (CVDs), diabetes, obesity, and healthcare costs is alarming. Consequently, there is an urgent need to identify cost-effective strategies for preventing CVDs, diabetes, and obesity. Accumulating evidence suggests that alterations in the gut microbiota, microbe-derived short-chain fatty acids, and trimethylamine play a crucial role in the pathophysiology of CVDs through the gut-brain-microbiota axis, which involves both the neural and immune systems. Consuming probiotics and prebiotics, as well as antioxidant diets rich in flavonoids, polyphenols, and carotenoids, while reducing the intake of salt, sugar-sweetened beverages, and saturated fats, quitting smoking, limiting excessive drinking, and engaging in moderate exercise for 30 minutes a day, are collectively recognized as effective strategies for preventing CVDs. This comprehensive approach is likely the most cost-efficient for promoting health and preventing chronic conditions such as cancer, diabetes, obesity, CVDs, and neurodegenerative diseases. Substantial evidence supports that the Mediterranean diet, which includes whole grains, omega-3 fatty acids, poultry, fish, nuts, seeds, olive oil, dairy products, limited red meat, and moderate red wine consumption, is associated with lower mortality and morbidity from CVDs. Additionally, recent studies suggest that dietary supplements containing flavonoids, carotenoids, and antioxidants can influence gene and protein expression, thereby altering metabolic pathways and maintaining body homeostasis, which in turn reduces the risk of chronic diseases with complex causes. The positive effects of plant-derived bioactive compounds and their metabolites arise from their combined antioxidant and anti-inflammatory properties. Probiotics and prebiotics contribute by promoting the healthy gut microbiota, enhancing gut endothelial integrity, and strengthening the body's immune function.

PREDIABETES IN ST-ELEVATION MYOCARDIAL INFARCTION PATIENTS: IMPLICATIONS FOR LEFT VENTRICULAR FUNCTION ONE YEAR POST-INFARCTION

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Background/Introduction: Prediabetes is associated with worse left ventricular (LV) ejection fraction (EF) in patients with previous ST-elevation myocardial infarction

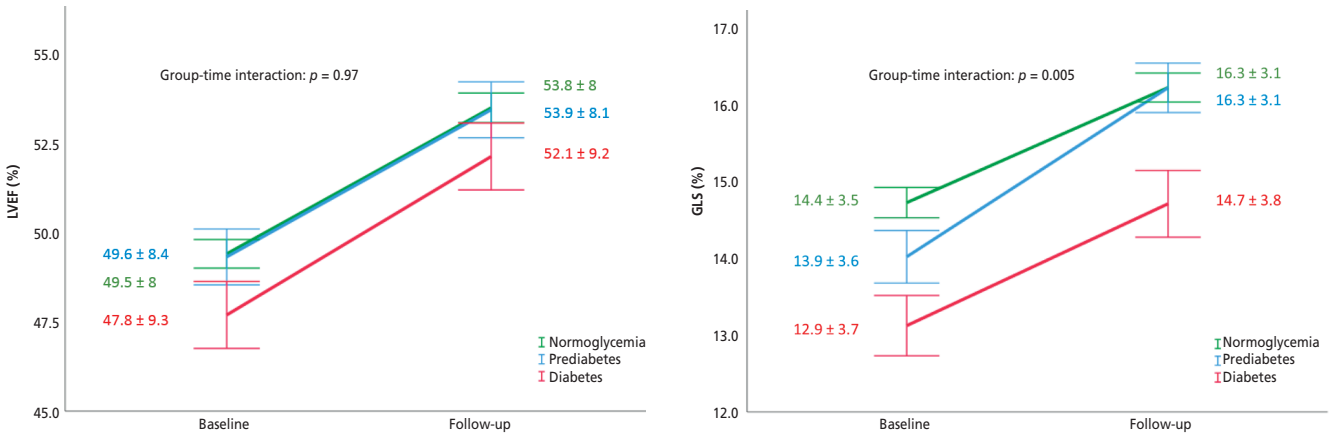

Fig. 1 – Left ventricular function change after ST-elevation myocardial infarction in normoglycemic, prediabetic and diabetic patients. LVEF – left ventricular ejection fraction; LVGLS – left ventricular global longitudinal strain.

(STEMI). However, the impact of prediabetes on changes in LV function in patients post-STEMI has not been evaluated.

Purpose: The aim of the study was to compare changes in LVEF and LV global longitudinal strain (GLS) one year after STEMI in patients with a wide spectrum of impaired glucose metabolism.

Table 1 – Effect size estimations for LVGLS at baseline and one year on multivariable linear mixed models

Variable	Effect size (95% CI)	*p*-value
Age, years	–0.01 (–0.02; 0.03)	0.13
Previous heart failure	–1.38 (–2.68; –0.08)	0.037
BMI, kg/m^2	–0.06 (–0.10; –0.03)	<0.001
Culprit lesion in LM or LAD	–1.85 (–2.09; –1.61)	<0.001
Multivessel disease	–0.33 (–0.57; –0.09)	0.007
Troponin, ng/l	–1.63 (–1.85; –1.42)	<0.001
eGFR, ml/min/1.73 m^2	0.01 (0.001; 0.02)	0.038
Heart rate, bpm	–0.05 (–0.06; –0.04)	<0.001
QRS >120 ms	–0.91 (–1.41; –0.41)	< 0.001
LVEDV, ml	–0.02 (–0.02; –0.02)	<0.001
IVST, mm	–0.07 (–0.14; –0.01)	0.024
Diastolic dysfunction	–0.78 (–1.13; –0.42)	<0.001
Normoglycemia	**Reference**	
Prediabetes	0.36 (–0.03; 0.75)	0.07
Diabetes	–0.72 (–1.10; –0.34)	<0.001
Normoglycemia	**Reference**	
Prediabetes – time interaction	0.75 (0.31; 1.18)	<0.001
Diabetes – time interaction	0.20 (–0.21; 0.60)	0.34

BMI – body mass index; bpm – beats per minute; eGFR – estimated glomerular filtration rate; IVST – interventricular septum thickness; LAD – left anterior descending coronary artery; LM – left main coronary artery; LVEDV – left ventricular end-diastolic volume; LVGLS – left ventricular global longitudinal strain.

Methods: Data were analyzed retrospectively from an ongoing large STEMI registry. Prediabetes was defined as glycated hemoglobin 5.7–6.4% in the absence of diabetes mellitus. Transthoracic echocardiography was performed within 48 hours of hospitalization and at one year follow-up. Patients were excluded if the glycemic status was unknown or changed at follow-up, or if follow-up data were unavailable. Changes in LVEF and LVGLS were compared between patients with normal glucose metabolism, prediabetes and diabetes mellitus. The effect sizes for covariables and group-time interactions were calculated using linear mixed models.

Results: Total of 1788 patients (mean age 60 ± 11 years; 1363 (76%) men), of whom 1126 (63%) had normal glucose metabolism, 284 (16%) had prediabetes and 378 (21%) had diabetes. During the index hospitalization normoglycemic and prediabetes patients had a similar LVEF (49.5 ± 8% vs 49.6 ± 8.4%) whereas LVEF was significantly worse in patients with diabetes (47.8 ± 9.3%; p <0.05 on Bonferroni post-hoc analysis). At one year follow-up, LVEF improved similarly across all the 3 groups (group-time interaction p 0.97, see **Figure 1**).

Baseline LVGLS differed significantly among the three groups, with the normoglycemic group having the highest LVGLS, while the diabetes group had the most reduced LVGLS (14.4 ± 3.5% in normoglycemic patients versus 13.9 ± 3.6% in prediabetic patients, versus 12.9 ± 3.7% in diabetic patients; p <0.05 with Bonferroni post-hoc analysis). LVGLS improved in all groups, but a significant group-time effect was observed in the patients with prediabetes, who improved more as compared to the patients with normoglycemia and diabetes (group-time interaction p = 0.005; see **Figure 1**). Differences remained significant after adjusting for relevant clinical and echocardiographic factors (see **Table 1**).

Conclusion: Prediabetes was associated with worse LVGLS but not LVEF after STEMI as compared to normoglycemia group, while the diabetes group had lowest LVGLS. At one year follow-up, LVGLS improved most in prediabetes group, reaching similar LVGLS values as normoglycemic patients. Differences in improvement of LVGLS remained significant after adjusting for relevant clinical and echocardiographic factors.

30-YEAR TRENDS IN BLOOD PRESSURE, PREVALENCE, AWARENESS, TREATMENT AND CONTROL OF HYPERTENSION IN WOMEN OF REPRODUCTIVE AGE. THE CZECH MONICA AND CZECH POST-MONICA STUDIES

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Women of reproductive age have not been included in clinical trials, therefore the limited information available comes from a few epidemiological studies.

Objective: To assess trends in BP levels and epidemiology of hypertension in women of reproductive age in representative population samples in the Czech Republic.

Design and methods: A total of seven independent cross-sectional population surveys of major CV risk factors were performed in 1985, 1988, 1992, 1997/1998, 2000/2001, 2007/2008 and 2015–2018 in a 1% random population sample. This analysis includes 4,913 women aged 25–44 years (mean age 35.6 ± 5.7 years). Hypertension was defined as a mean of 2 BP readings ≥ 140/90 mmHg taken at one visit or being on antihypertensive medication. Hypertension was considered controlled if BP was < 140/90 mmHg (in individuals treated by antihypertensive drugs). Detailed information on treatment is available only since the fourth survey.

Results: Over a period of 32 years, there was a significant reduction in both systolic BP (from 121.3 ± 15.5 to 117.2 ± 13.7 mmHg; p <0.001) and diastolic BP (from 78.2 ± 10.1 to 77.2 ± 9.2 mmHg; p <0.001). The prevalence of hypertension decreased from 19.3% to 11.0% (p <0.001) with no significant changes in BMI. There was also an increase in the awareness of hypertension (from 42.2% to 66.1%; p <0.01) and the proportion of individuals treated by antihypertensive drugs (from 14.1% to 37.3%; p <0.001). Hypertension control improved (from 3.1% to 25.4%; p <0.001). Most of the women were treated only by one antihypertensive drug (raging between 63.3% and 59.1%), during the third and fourth survey mostly by beta-blockers, replaced later by RAS blockers.

Conclusions: Despite significant improvement in all epidemiological parameters, the control rates remain poor, mostly due to a low proportion of women treated by antihypertensive drugs and a low usage of combination therapy. This might be a reflection of a wrong perception that these women are at low risk.

MOLECULAR CHANGES IN CALCIUM HANDLING ASSOCIATED WITH INCREASED ARRHYTHMIA SUSCEPTIBILITY IN A LARGE ANIMAL MODEL OF INTENSIVE TRAINING

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Introduction: Intensive training in elite athletes is associated with a greater prevalence of both atrial and ventricular arrhythmias. Our previous results have revealed that four months of high-intensity training in dogs increased susceptibility to ventricular fibrillation *in vivo*. In trained animals, we observed higher action potential and calcium alternans, increased Purkinje–ventricle APD dispersion, reduced calcium influx and sarcoplasmic reticulum (SR) calcium content, slower calcium relaxation kinetics, and larger calcium buffering compared to sedentary controls. Here we investigated the expression of key calcium-handling proteins, potentially contributing to arrhythmogenic remodeling in a canine model of intensive training.

Methods: Protein expression levels of $Ca_v1.2$, NCX1, SERCA2, and CASQ2 were assessed by Western blotting and immunocytochemistry in left ventricular tissue and isolated cardiomyocytes from both sedentary and trained dogs.

Results: In trained animals, $Ca_v1.2$ expression was significantly downregulated, while NCX1, SERCA2, and CASQ2 levels remained unchanged. In this model, reduced calcium influx and SR calcium content-alongside preserved cell shortening, slower calcium relaxation kinetics, and increased calcium buffering occurred in cardiomyocytes. Additionally, on protein level selective downregulation of $Ca_v1.2$ was observed.

Conclusions: Preserved SERCA2 and CASQ2 protein expression may reflect a downregulated, yet energetically efficient calcium-handling. This selective decrease in $Ca_v1.2$ may create an imbalance between calcium influx and cycling, promoting electrical instability and increased arrhythmia susceptibility.

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ADVANCES IN MSC THERAPY: BENCH TO BEDSIDE

Dawn B

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Despite intense research over the past several decades, the efficacy of mesenchymal stem cells (MSCs) in therapeutic organ repair continues to remain experimental. Data from animal models and clinical trials indicate that MSCs harvested from various tissues induce cardiac repair. Promising results from clinical trials also indicate that MSC therapy is associated with improved outcomes. Consistently, the number of clinical trials with various MSCs have increased significantly in recent years and several MSC products have been approved worldwide for human use. Nonetheless, a number of challenges remain to be overcome in order to realize the full potential of MSC therapy in clinical practice. The purpose of this talk is to summarize the evidence regarding the translation of MSC therapy for regenerative purposes.

EFFECT OF CONVENTIONAL AND NEW TYPE OF INOTROPIC AGENTS ON MITOCHONDRIAL RESPIRATION

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Introduction: In heart failure with reduced ejection fraction, the aim is to increase cardiac output and relieve symptoms. Although positive inotropic agents improve hemodynamic parameters, they have a negative impact on survival, this is particularly true for conventional catecholamines that raise intracellular Ca^{2+} levels. The goal of our study was to investigate whether there is a correlation between the effects of traditional and newer types of positive inotropic agents on mitochondrial function and patient survival.

Methodology: We used an Agilent Seahorse XFp analyzer to measure mitochondrial respiration in H9c2 cell cultures. This method enables real-time, non-invasive assessment of cellular bioenergetics by quantifying the oxygen consumption rate (OCR). This parameter reflects key aspects of mitochondrial function, including basal respiration, ATP-linked respiration, maximal respiratory capacity, and spare respiratory capacity. To complement this, mitochondrial membrane potential was evaluated using JC-1 staining, a potentiometric fluorescent dye that differentiates between polarized and depolarized mitochondria based on dye aggregation.
Results: Of the conventional catecholamines, dobutamine dose-dependently reduced mitochondrial basal respiration, an effect not observed with dopamine. Levosimendan did not significantly impair mitochondrial oxygen consumption, whereas its active metabolite at low concentrations enhanced maximal respiration. The myosin activator omecamtiv mecarbil was ineffective on mitochondrial function in non-pulsating cells. Istaroxime enhanced cellular respiration at low doses and significantly impaired it at high doses.
Conclusion: Our studies suggest that the negative effects of dobutamine on mitochondrial metabolism may contribute to poor survival. Furthermore, the improvement in mitochondrial function observed of istaroxime may play a role in the promising clinical results.

MODIFICATION OF ISCHEMIA--REPERFUSION INJURY BY POLYCLONAL ANTIBODIES AGAINST SARCOLEMMAL CA^{2+}-ECTO ATPASE

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Ischemia-reperfusion injury has been shown to be associated with depressed recovery of cardiac contractile function, development of oxidative stress and occurrence of intracellular Ca^{2+}-overload. However, the mechanisms for Ca^{2+}-entry into cardiomyocytes are poorly understood. We have identified the presence of Ca^{2+}-ecto ATPase in the sarcolemmal membrane and have shown it to be stimulated by oxidative stress. We have also provided the evidence that this enzyme is activated by millimolar concentrations of Ca^{2+} and serves as a Ca^{2+}-gating mechanism for Ca^{2+}-entry in cardiomyocytes. We have now observed that polyclonal antibodies against the purified Ca^{2+}-ecto ATPase improved the recovery of cardiac function of the ischemic reperfused heart. Furthermore, these antibodies depressed the ATP-induced increase in intracellular Ca^{2+} without affecting the KCl or Bay-8644 induced increase in Ca^{2+} in cardiomyocytes. These observations support the

view that Ca2+-ecto ATPase may be involved in promoting Ca^{2+}-entry, intracellular Ca^{2+}-overload and cardiac dysfunction due to ischemia-reperfusion injury in the heart.

CARDIOPROTECTIVE EFFECTS OF PYRIDOXINE IN EXPERIMENTAL DIABETIC CARDIOMYOPATHY AND HYPERHOMOCYSTEINEMIA: EVALUATION ON CARDIOMETABOLIC STATUS, OXIDATIVE STRESS AND HISTOMORPHOLOGICAL CHANGES

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The first part of this study was to examine the effects of pyridoxine (vitamin B_6) administration on cardiometabolic biomarkers, activities of cardiac antioxidant stress enzymes superoxide dismutase (SOD) and catalase (CAT), and enzyme indicators of cardiometabolic status, lactate and malate dehydrogenase (LDH, MDH), as well as LDH and MDH isoforms' distribution in the cardiac tissue of healthy and diabetic Wistar albino male rats. Experimental animals were divided into five groups: C1 – control (0.9% sodium chloride – NaCl – 1 mL/kg, intraperitoneally (i.p.), 1 day); C2 – second control (0.9% NaCl 1 mL/kg, i.p., 28 days); DM – diabetes mellitus (streptozotocin 100 mg/kg in 0.9% NaCl, i.p., 1 day); P – pyridoxine (7 mg/kg, i.p., 28 days); and DM + P – diabetes mellitus and pyridoxine (streptozotocin 100 mg/kg, i.p., 1 day and pyridoxine 7 mg/kg, i.p., 28 days). The second part was to examine the effects of pyridoxine administration on cardiometabolic biomarkers, oxidative stress parameters, metabolic enzymes activities and histomorphological changes in cardiovascular system of rats (the heart and aorta) in hyperhomocysteinemic conditions. Wistar albino male rats were divided into four groups (n = 10, per group): C: 0.9% NaCl 0.2 mL/day s.c. + 0,9% NaCl 0.5 mL i.p; H: D,L-homocysteine 0.45 mmol/g b.w./day s.c. + 0.9% NaCl 0.5 mL i.p; CB6: 0.9% NaCl 0.2 mL/day s.c. + vitamin B_6 7 mg/kg b.w. i.p.; and H-B6: D,L-homocysteine 0.45 mmol/g b.w./day s.c. + vitamin B_6 7 mg/kg b.w. i.p. (substances were applied s.c. for 2 weeks and i.p for 4 weeks). Pyridoxine treatment reduced CAT and MDH activity in diabetic rats. In diabetic rats, the administration of pyridoxine increased LDH1 and decreased LDH4 isoform activities, as well as decreased peroxisomal MDH and increased mitochondrial MDH activities. Four-week treatment with vitamin B_6 in experimentally induced hyperhomocysteinemia decreased serum values of tHcy, and decreased oxidative stress level in cardiac tissue. The results showed the positive effects of pyridoxine administration on the complex interplay between oxidative stress, antioxidant enzymes, and cardiometabolic status in experimentally induced diabetic cardiomyopathy as well as in hyperhomocysteinemia in rats.

PROGNOSTIC SIGNIFICANCE OF ADAPTIVE VS. MALADAPTIVE RIGHT VENTRICULAR HYPERTROPHY IN HEART FAILURE: A PROSPECTIVE COHORT STUDY

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Background: Right ventricular (RV) dysfunction is a key driver of poor outcomes and increased mortality among patients with heart failure (HF). In this population, pulmonary hypertension is the predominant contributor to RV dysfunction, and the primary compensatory mechanism is RV hypertrophy (RVH). Evidence from other pulmonary hypertension cohorts suggests that maladaptive RVH is linked to worse clinical outcomes and higher rates of RV dysfunction. However, the prognostic implications of adaptive RVH patterns in HF remain unclear. This study sought to evaluate the short-term outcomes of patients with adaptive and maladaptive RVH phenotypes.
Methods: We prospectively enrolled 195 HF patients from eight centers. Adaptive RVH was defined as RV free wall thickness ≥5 mm with RV minor diameter ≤41 mm, whereas maladaptive RVH was defined as RV minor diameter >42 mm. All participants were followed for six months for the composite endpoint of all-cause mortality and HF-related hospitalizations.
Results: Patients with maladaptive RVH experienced significantly higher rates of the composite endpoint compared with those with adaptive RVH or normal RV dimensions (log-rank $p = 0.004$). Pulmonary artery pressure, male sex, atrial fibrillation, and elevated creatinine levels were independent predictors of maladaptive RVH. Although patients with adaptive RVH showed outcomes comparable to those with normal RV (pairwise $p = 1$), a subgroup with more severe RVH (free wall thickness ≥6.1 mm) exhibited a higher event rate (log-rank $p = 0.006$).

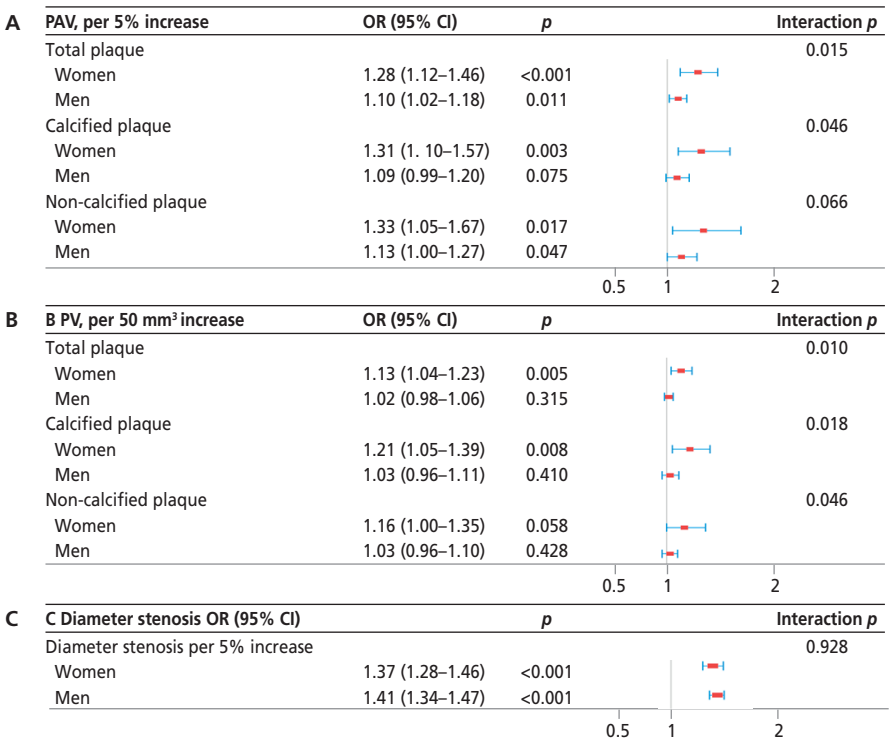

Fig. 1 – The association between ischemia and PAV (A), PV (B) and diameter stenosis (C) in women and men. * Model adjusted for age ≥65 years, diabetes mellitus, vessel type, diameter stenosis, ≥2 HRP characteristics. CI – confidence interval; OR – odds ratio; PAV – percentage atheroma volume; PV – plaque volume.

Conclusions: Maladaptive RV hypertrophy is associated with an adverse short-term prognosis in HF patients. More severe adaptive RVH may also carry a higher risk of adverse outcomes

SEX BASED DIFFERENCES IN ASSOCIATION WITH PLAQUE BURDEN AND ISCHEMIA – A CREDENCE TRIAL SUBSTUDY

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Background: Prior studies have identified sex-based differences in coronary computed tomography angiography (CCTA)-derived plaque characteristics and their association with clinical outcomes. Women typically exhibit a less severe stenosis and a lower overall plaque burden; however, this does not correspond to a proportionally lower rate of adverse clinical events. The relationship between coronary plaque burden and ischemia, as defined by invasive fractional flow reserve (FFR), across sexes remains unclear.
Purpose: This study aimed to investigate sex-based differences in quantitative plaque burden assessed by CCTA and their relationship to ischemia, as determined by invasive FFR.
Methods: A post hoc analysis of the CREDENCE (Computed Tomographic Evaluation of Atherosclerotic Determinants of Myocardial Ischemia) trial was conducted, involving 612 symptomatic patients (184 women and 428 men) with suspected stable coronary artery disease. All participants underwent CCTA and invasive coronary angiography with FFR measurements. Quantitative plaque analysis assessed plaque volume and burden of total, calcified, and non-calcified plaque (TP, CP and NCP). Ischemia was defined as invasive FFR ≤0.8.
Results: Women were generally older (66.5 ± 9.1 years vs. 63.5 ± 10.3 years, p = 0.001) and had lower prevalence of smoking. Other clinical risk factors, including angina and medication use, were comparable between men and women.
CCTA findings revealed that women had a lower prevalence of high-risk plaques, shorter lesion length, and a less severe stenosis. Quantitative plaque analysis showed that women had significantly lower plaque volume and burden across all components (all p <0.05), except for calcified plaque burden, which was similar between sexes (2.60% vs. 3.00%, p = 0.166).
In the multivariable analysis, a per 5% increase in plaque burden was significantly associated with ischemia in women for TP (OR = 1.28, 95% CI [1.12–1.46], p <0.001), CP (OR = 1.31, 95% CI [1.10–1.57], p = 0.003), and NCP (OR = 1.33, 95% CI [1.05–1.67], p = 0.017). In men, only the plaque burden of TP and NCP, but not CP showed

significant associations. A significant interaction between sex and plaque burden was observed for TP (p = 0.015) and CP (p = 0.046). After adjusting for variables shown in the **Figure 1**, a significant interaction between sex and plaque volume was noted in the PV of TP, CP, and NCP (all p <0.05), indicating a stronger association between plaque and ischemia in women versus men. Of note, interactions between sex and diameter stenosis were not significant (p = 0.928).
Conclusion: Women exhibit a lower overall plaque burden compared to men, yet the association between plaque burden and ischemia is more pronounced in women. These findings suggest that even modest increase in plaque burden may have greater ischemic implications for women, underscoring the importance of sex-specific diagnostic and therapeutic strategies in managing coronary artery disease.

THE EFFECT OF PREVENTIVE AND THERAPEUTIC ADMINISTRATION OF CURCUMIN ON OXIDATIVE STRESS IN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS

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Introduction: Rheumatoid arthritis (RA) is chronic, progressive autoimmune disease characterized by inflammation and progressive disability in synovial joints. Oxidative stress is significant pathogenic mechanism responsible for the initiation and maintenance of pathophysiological processes in the development of RA. Curcumin, polyphenol from turmeric (*Curcuma longa*), is a potent scavenger of variety reactive oxygen species with antioxidant activity. The aim of this study was to investigate antioxidant activity of curcumin in treatment of RA and its potential preventive effect, alone or in combination with methotrexate.
Materials and methods: RA was induced by administering 0.1 ml of Complete Freund's Adjuvant (CFA) subcutaneously at the base of the *Wistar albino* rat tail. In this study, 48 rats were divided into eight groups: 1. CTRL: negative control, 2. CUR: positive control 1 (curcumin 200 mg/kg three times a week for 4 weeks per os), 3. MTX: positive control 2 (methotrexate 0,75 mg/kg i.p. two times a week for 4 weeks), 4. RA: positive control 3 (CFA-induced RA), 5. RA+pCUR: rats with CFA-induced RA + preventive administration of curcumin (curcumin 200 mg/kg three times a week for 4 weeks per os before the induction of RA), 6. RA+tCUR: rats with CFA-induced RA + curcumin therapy, 7. RA+MTX+pCUR; rats with CFA-induced RA + preventive administration of curcumin + methotrexate 0.75 mg/kg i.p. twice weekly for 4 weeks, 8. RA+MTX+tCUR: rats with CFA-induced RA + therapeutic administration of curcumin + methotrexate 0.75 mg/kg i.p. twice weekly for 4 weeks. Blood samples were collected 28 days after induction of RA, at the time of animal sacrifice. Parameters of oxidative stress, pro-oxidants and enyzmes of antioxidant defense, were determined from blood plasma and erythrocyte lysates by spectrophotometric method.
Results: Preventive and therapeutic administration of curcumin reduced the level of pro-oxidants and improved antioxidant capacity by increasing activity of antioxidant enzymes in rats with CFA-induced RA. Combining curcumin and methotrexate has a synergistic effect in mitigating oxidative stress in rats with RA.
Conclusion: Curcumin has a beneficial effect in the treatment of RA due to its antioxidant properties. These results imply consideration of potential application of curcumin to reduce oxidative stress in RA patients.

THE EFFECT OF SGLT2 INHIBITORS ON SECRETONEURIN LEVELS IN HEART FAILURE PATIENTS: A SIX-MONTH FOLLOW-UP

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Introduction: SGLT2 inhibitors (SGLTi) represent a significant advancement in heart failure management due to their favorable impact on cardiovascular outcomes. Secretoneurin (SCN) is a biomarker linked to neurohumoral activation and could serve as an indicator of clinical status in heart failure patients. This study evaluates the impact of SGLTi on SCN dynamics over a six-month follow-up period.
Aim: To assess whether the use of SGLT2 inhibitors influences secretoneurin levels in heart failure patients.
Methods and patient cohort: Fifty-one heart failure patients were enrolled in the study and divided based on SGLTi use: 37 patients on SGLTi and 14 not on SGLTi. Secretoneurin levels were measured at baseline (SCN-1), after 3 months (SCN-2), and after 6 months (SCN-3). Data were analyzed using descriptive statistics and group comparisons.
Results: The mean SCN values in the SGLTi group showed a decrease over time (SCN-1: 40.4 ± 29.1 ng/L; SCN-2: 47.6

± 24.7 ng/L; SCN-3: 56.9 ± 27.9 ng/L), while the non-SGLTi group showed less pronounced changes. Although the differences between the groups were not statistically significant (p >0.05), a trend toward a more favorable SCN profile was observed in the SGLTi group.
Conclusion: Heart failure patients treated with SGLT2i showed a trend of improved SCN levels over time. Although the differences between groups were not statistically significant, these findings suggest a potential benefit of SGLTi on neurohumoral activation in heart failure, warranting further research with a larger cohort.

BIOMARKERS

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Background: Secretoneurin (SCN) is a promising biomarker reflecting neurohumoral activity in heart failure with reduced ejection fraction (HFrEF). Its temporal dynamics may be influenced by pharmacotherapy, particularly angiotensin-converting enzyme inhibitors (ACEI) and angiotensin receptor-neprilysin inhibitors (ARNI).
Methods: To evaluate the effect of ACEI and ARNI therapy on SCN levels over time (baseline, 3 months, 6 months) in HFrEF patients. A secondary objective was to assess the influence of additional clinical and laboratory factors on SCN levels.
We performed a retrospective analysis of 51 HFrEF patients, all with ICD implants. Patients were divided into two groups based on their treatment regimen (ACEI vs. ARNI). SCN levels were measured at baseline, 3 months, and 6 months. Additional factors such as age, BMI, NT-proBNP, EF, and comorbidities (e.g., diabetes) were evaluated. Statistical analysis included paired t-tests and ANOVA for group comparisons over time.
Mean age: 65 ± 12 years, 76% male, mean LVEF: 28 ± 6%, therapy distribution: ACEI: 24 patients, ARNI: 27 patients.
Results: 1. SCN dynamics: In the ACEI group, SCN reduction at 6 months was not statistically significant (p = 0.08). In the ARNI group, a significant reduction in SCN levels was observed after 6 months (p <0.01).
2. Subanalysis: Patients with higher baseline NT-proBNP exhibited a greater reduction in SCN (p <0.05). Diabetes mellitus was associated with consistently higher SCN levels across all time points, irrespective of therapy. SCN correlated positively with age and serum creatinine (r = 0.35; p <0.05).
3. Medication comparison: ARNI therapy resulted in significantly greater SCN reduction compared to ACEI over 6 months (p <0.01).
Conclusion: ARNI therapy significantly decreases SCN levels in HFrEF patients compared to ACEI. SCN emerges as a potential biomarker for therapy response in HFrEF. Additional factors such as NT-proBNP, diabetes, and renal function further influence SCN dynamics, underscoring the need for personalized therapeutic strategies.

EVALUATION OF CHENODEOXYCHOLIC ACID AS A CARDIOPROTECTIVE AGENT IN ISOPRENALINE-INDUCED MYOCARDIAL INJURY

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Introduction: Hydrophilic bile acids like ursodeoxycholic acid (UDCA) have demonstrated significant cardioprotective effects in preclinical models, due to their cytoprotective and anti-apoptotic properties. In contrast, chenodeoxycholic acid (CDCA) is considered hydrophobic and has traditionally been associated with cytotoxicity at high concentrations. However, CDCA is the least hydrophobic among hydrophobic bile acids, suggesting a potential for beneficial cardiovascular effects under controlled conditions. This study aimed to explore whether CDCA could confer cardioprotection in a model of isoprenaline-induced myocardial injury in rats.

Methodology: Male Wistar albino rats were randomly assigned to four groups and pre-treated via oral gavage for 10 days: propylene glycol + saline s.c. on days 9 and 10 (control group), propylene glycol + isoprenaline s.c. on days 9 and 10 (I group), CDCA + saline s.c. on days 9 and 10 (CDCA group), and CDCA + isoprenaline s.c. on days 9 and 10 (CDCA + I group). CDCA was dissolved in propylene glycol and administered at 25 mg/kg, while isoprenaline was given subcutaneously at 85 mg/kg. Animals were sacrificed 24 hours after the final treatment for biochemical and histological analyses.
Results: CDCA pretreatment significantly reduced high-sensitivity troponin I (hsTnI) levels, suggesting myocardial protection, and showed a trend toward decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as cardiac injury markers. CDCA also reduced thiobarbituric acid reactive substances (TBARS) (p <0.01), increased glutathione (GSH) levels, and showed a tendency to elevate superoxide dismutase (SOD) and catalase (CAT) activity. CDCA lowered the expression of tumor necrosis factor-alpha (TNF-α), indicating an anti-inflammatory effect. However, pathohistological analysis did not reveal clear structural improvement in cardiac tissue.
Conclusion: CDCA demonstrated biochemical signs of cardioprotection by reducing myocardial injury markers, oxidative stress, and inflammation, but pathohistological analysis showed no clear improvement in cardiac tissue structure. Additional studies are needed to elucidate the molecular mechanisms related to cardioprotective effects of CDCA.

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RISK FACTORS FOR SPINAL ANESTHESIA – INDUCED HYPOTENSION DURING THE ELECTIVE CESAREAN SECTION

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Introduction: Spinal anesthesia is widely regarded as the "gold standard" for elective cesarean sections due to its rapid onset and the reduced risk of complications associated with general anesthesia. However, hypotension remains the most common side effect, with its incidence influenced by maternal and procedural factors. Despite the use of preventive strategies and ongoing research into dose adjustments based on patient characteristics, predictive accuracy remains limited. This study aimed to explore the relationship between hypotension and patient variables including age, BMI, height, preoperative fasting and hypertension during pregnancy.
Methodology: This retrospective study analyzed medical records of 123 patients who underwent elective cesarean section under spinal anesthesia between April and August 2023 at the Clinic of Gynecology and Obstetrics, Novi Sad, Serbia. Demographic data, anesthetic dose, timing of surgery, and blood pressure trends were collected. Patients were grouped by delivery time (8–10 AM, 10–12 AM, after 12 PM), height (≥165 cm or <165 cm), hypotension occurrence (systolic BP <90 mmHg), and hypertension history. Statistical analysis included t-test, Mann–Whitney U, chi-square, ANOVA, and correlation testing, with p <0.05 considered significant.
Results: Hypotension occurred in 56 (45.5%) patients. Older age was associated with hypotension (p = 0.031), while hypertension in pregnancy was linked to a lower hypotension incidence (p = 0.032). Height, BMI, and surgery timing did not significantly influence hypotension risk. Taller patients received higher anesthetic volumes (p = 0.005), though dose did not predict hypotension. A moderate correlation between height and anesthetic volume was found (rho = 0.427, p <0.001). Most hypotensive episodes occurred within 10 minutes post-anesthesia. No significant differences were observed in vasopressor use or hypotension frequency by height. Anesthetic volume did not correlate with blood pressure drop magnitude.
Conclusion: Hypotension was associated with older age, while hypertension during pregnancy appeared protective. No associations were found with height, BMI, or fasting duration. Findings highlight the need for individualized monitoring and larger-scale studies to optimize spinal anesthesia management in cesarean delivery.

NECROSIS-LIKE CELL DEATH LIKELY PLAYS AN IMPORTANT ROLE IN HEART FAILURE WITH REDUCED BUT NOT PRESERVED EJECTION FRACTION: MECHANISMS AND THERAPEUTIC APPROACHES

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Active necrosis-like cell death has been shown to underlie cardiac damage due to acute myocardial infarction and plays an important role in the phenotypes of heart failure with reduced ejection fraction. Indeed, both necroptosis and pyroptosis which are linked with the activation of the innate immune system and inflammation, have been reported in such damaged hearts while failing hearts with preserved ejection fraction are negative for the necroptotic markers advocating for no necroptotic environment. In contrast, blocking of RIP3, a protein kinase with multiple action besides promoting the necroptotic signalling, has been shown to mitigate cardiac damage and prevent mitochondrial swelling in hearts damaged due to ischemia. Such beneficial effects are associated with the reduced release of damage-associated molecular patterns (e.g. HMGB1, and mitochondrial DNA) indicating the retardation of the further deleterious, pro-inflammatory intracellular and extracellular signalling pathways. Likewise, the anti-necroptotic treatment has been suggested to reduce the higher circulating levels of RIP3 in subjects with heart failure. In summary, pro-death events resembling necrotic features might be of a great interest for diagnosis of heart failure with reduced ejection fraction and for designing of cardioprotective agents.

BIORESORBABLE ELECTRONIC DEVICES FOR CARDIAC PACING AND DEFIBRILLATION

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Temporary pacemakers are essential for the care of patients with short-lived bradycardia in post-operative and other settings. Conventional devices require invasive open-heart surgery or less invasive endovascular surgery, both of which are challenging for pediatric and adult patients. Complications include risks of infections, lacerations, and perforations of the myocardium, and displacements of external power supplies and control systems. We have developed a millimetre-scale bioresorbable optoelectronic system with an onboard power supply and a wireless, optical control mechanism with generalized capabilities in electrotherapy and specific application opportunities in temporary cardiac pacing. The extremely small sizes of these devices enable minimally invasive implantation, including percutaneous injection and endovascular delivery. IT can also be integrated into transcatheter aortic valve replacement (TAVR) and similar devices associated with AV block. Experimental studies demonstrate effective pacing in mouse, rat, porcine, canine, and human cardiac models at both single-site and multi-site locations. Pairing with a skin-interfaced wireless device allows autonomous, on-demand pacing upon detection of arrhythmias. Further work illustrates opportunities in combining these miniaturized devices with other medical implants, such as arrays of pacemakers for individual or collective use on the frames of TAVR systems, to provide unique solutions that address the risks of atrioventricular block following surgeries.

MORE MITOCHONDRIAL FISSION MEDIATOR DRP1 IN ENDOTHELIAL CELLS – INCREASED MICROVASCULAR DAMAGE AFTER ISCHEMIA/REPERFUSION INJURY

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Background: In recent decades, research on myocardial ischemia/reperfusion (I/R) injury has focused on cardiomyocytes. However, both cardiomyocytes and coronary arteries, especially the microvasculature, are injured during acute myocardial infarction. Microvascular damage has been shown to affect patients' prognosis, independent of infarct size. In cardiomyocytes, changes in mitochondrial dynamics is one response to I/R injury, and this shift in mitochondrial dynamics is thought to contribute to cardiomyocyte I/R injury. Preliminary in vitro studies in endothelial cells established a link between mitochondrial dynamics and I/R-induced endothelial cell damage. However, it remains unclear whether mitochondrial dynamics in endothelial cells influence microvascular (dys)function after I/R. Therefore, we aimed to investigate the effect of an increased endothelium-specific mitochondrial fission mediator dynamin-related protein 1 (Drp1) expression on infarct size and microvascular damage (no-reflow).
Methodology: Isolated perfused hearts from endothelial-specific Drp1-overexpressing (eDRP1+; n = 14) or littermate control (control; n = 10) Sprague-Dawley rats were subjected to 30 min regional ischemia by occlusion of the left anterior descending coronary artery followed by 120 min reperfusion. Left ventricular developed pressure (LVDP) and coronary flow (CF) were measured. Patent blue was used to delineate the area at risk, triphenyl-tetrazolium-chloride to stain infarct size, and thioflavin-S to visualize microvascular injury.
Results: LVDP and CF were similar at baseline (88 ± 8 vs. 92 ± 10 mmHg, $p = 0.314$; 16.4 ± 2.0 vs 15.8 ± 1.3 mL/min, $p = 0.423$) and 10 min reperfusion (74 ± 26 vs. 70 ± 28 mmHg, $p = 0.703$; 15.1 ± 2.0 vs. 15.6 ± 1.5 mL/min, $p = 0.471$) between eDRP1+ and -control hearts. Area at risk (51 ± 8 vs. 48 ± 7% left ventricle; $p = 0.524$) and infarct size (44 ± 10 vs. 45 ± 9% area at risk, $p = 0.663$) were comparable between

eDRP1+ and -control hearts. The area of no-reflow was increased in eDrp1+ compared to the eDRP1-control hearts (7.3 ± 5.3 vs. 3.1 ± 2.5% area at risk, $p = 0.030$).
Conclusion: Our results provide the first evidence of a causal relationship between the shift in endothelial mitochondrial dynamics towards increased mitochondrial fission and microvascular damage by myocardial I/R. This suggests that mitochondria might be a promising target for vasoprotection and thus may be cardioprotection.

IMPACT OF RIGHT VENTRICLE TO PULMONARY ARTERY COUPLING ON OUTCOMES IN PATIENTS WITH PRIMARY MITRAL REGURGITATION

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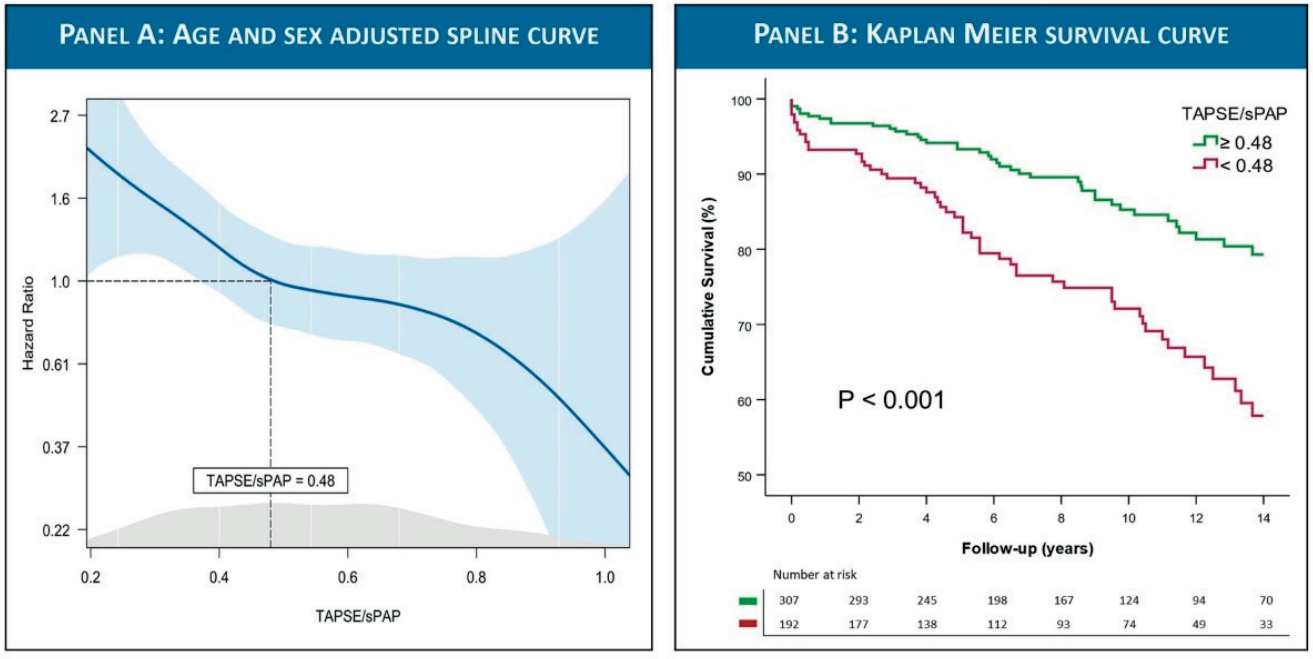
Background: In patients with significant primary mitral regurgitation (MR), both elevated pulmonary pressure and right ventricular (RV) dysfunction have been shown to be associated with adverse outcomes. However, the adaptation of the RV to increasing pulmonary pressures can vary, even within ranges of RV function that are considered normal. The concept of coupling the RV to pulmonary artery (PA) has therefore been introduced to better describe the match between the RV contractility and the opposing afterload, but has not been assessed so far in primary MR patients.
Purpose: To evaluate the association of RV-PA coupling with outcome in primary MR patients undergoing mitral valve surgery.
Methods: RV-PA coupling was measured before surgery as the ratio of tricuspid annular plane systolic excursion (TAPSE) to systolic pulmonary artery pressure (sPAP) in 499 patients with primary MR and elevated sPAP (≥30 mmHg by echocardiography). The study outcome was all-cause mortality.
Results: Based on age and sex adjusted spline curve analysis (**Fig. 1A**), TAPSE/sPAP values <0.48 mm/mmHg indicated RV/PA uncoupling and were associated with mortality hazard ratios >1. Compared to patients with a coupled RV, those with RV/PA uncoupling (192; 38.5%) were significantly older (mean age 70 vs 65 years; p <0.001), more symptomatic (88% vs 68%; p <0.001) and had higher rates of atrial fibrillation (54% vs 31%; p <0.001).
After a median follow-up of 8.3 years, 98 (19.6%) patients died. Kaplan–Meier analysis showed a significantly lower survival in patients with RV/PA uncoupling (70.8% vs 86.3%; p <0.001) (**Fig. 1B**). On multivariable analysis, impaired TAPSE/sPAP was independently associated with mortality (HR: 1.97; p = 0.015) after adjustment for significant clinical and echocardiographic covariates (**Table 1A**). To compare the predictive value



Panel (A): Determination of optimal TAPSE/sPAP cutoff using Cox proportional hazards. The blue line represents the age and sex adjusted hazard ratio for all-cause mortality, and the shaded blue areas represent the 95% confidence interval. Values < 0.48 were associated with hazard ratio > 1. **Panel (B):** Kaplan-Meier survival curve demonstrating the association between TAPSE/sPAP ratio and all cause mortality. Survival rate at 14 years was significantly lower in patients with impaired coupling ratio. SPAP = systolic pulmonary artery pressure; TAPSE = tricuspid annular plane systolic excursion.

Fig. 1 – TAPSE/sPAP cutoff and survival.

Table 1 – Multivariable regression analysis

TABLE A: MULTIVARIABLE COX REGRESSION ANALYSIS

	UNIVARIABLE		MODEL A		MODEL B		MODEL C	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age at surgery	**1.09 (1.06 – 1.12)**	**<0.001**	1.03 (1.00 – 1.07)	0.087	1.03 (0.99 – 1.07)	0.107	1.03 (0.99 – 1.07)	0.119
Female sex	1.38 (0.93 – 2.05)	0.113						
NYHA functional class ≥ II	**2.28 (1.25 – 4.18)**	**0.007**	1.00 (0.46 – 2.16)	0.999	0.99 (0.46 – 2.13)	0.988	0.97 (0.45 – 2.08)	0.941
Previous cardiac surgery	1.59 (0.58 – 4.33)	0.365						
Glomerular filtration rate	**0.97 (0.96 – 0.98)**	**<0.001**	0.99 (0.97 – 1.01)	0.231	0.99 (0.97 – 1.01)	0.224	0.99 (0.98 – 1.01)	0.268
Diabetes Mellitus	1.02 (0.32 – 3.22)	0.972						
Hypertension	1.28 (0.86 – 1.91)	0.220						
Atrial fibrillation	**2.05 (1.38 – 3.06)**	**<0.001**	1.64 (0.96 – 2.81)	0.072	1.46 (0.85 – 2.53)	0.174	1.54 (0.90 – 2.63)	0.115
Coronary artery disease	1.31 (0.84 – 2.04)	0.229						
Concomitant TV repair	0.86 (0.58 – 1.28)	0.466						
LV end-systolic diameter	1.00 (0.97 – 1.03)	0.850						
LV end-systolic volume	0.99 (0.98 – 1.00)	0.128						
LV ejection fraction	1.00 (0.97 – 1.02)	0.697						
Left atrial volume index	1.00 (1.00 – 1.01)	0.261						
Average e'	**0.81 (0.73 – 0.90)**	**<0.001**	**0.84 (0.76 – 0.94)**	**0.001**	**0.85 (0.76 – 0.94)**	**0.002**	**0.84 (0.76 – 0.94)**	**0.001**
RV basal diameter	1.00 (0.97 – 1.02)	0.790						
SPAP	**1.02 (1.00 – 1.03)**	**0.011**	1.02 (1.00 – 1.03)	0.110				
TAPSE	**0.92 (0.88 – 0.96)**	**<0.001**			**0.94 (0.89 – 1.00)**	**0.033**		
Impaired TAPSE/sPAP	**2.33 (1.56 – 3.48)**	**<0.001**					**1.97 (1.14 – 3.40)**	**0.015**
EROA	1.00 (0.99 – 1.01)	0.812						
Regurgitant volume	1.00 (0.99 – 1.01)	0.526						

TABLE B: COMPARISON BETWEEN MULTIVARIABLE MODELS

	BASE MODEL	MODEL A (+ sPAP)	MODEL B (+ TAPSE)	MODEL C (+ TAPSE/sPAP)
HR (P-VALUE)		1.015 (0.110)	**0.940 (0.033)**	**0.110 (0.009)**
STANDARDIZED HR		4.963	0.118	0.072
LIKELIHOOD RATIO (P-VALUE)		2.436 (0.119)	**4.621 (0.032)**	**7.417 (0.006)**
C-INDEX (95% CI)	0.726 (0.655 to 0.798)	0.730 (0.663 to 0.798)	0.734 (0.663 to 0.805)	0.741 (0.676 to 0.807)
NRI (95% CI)		0.245 (-0.103 to 0.549)	0.390 (-0.059 to 0.692)	**0.475 (0.102 to 0.846)**

Table (A): COX regression models for all-cause mortality. . The base model included age, NYHA class ≥ II, eGFR, atrial fibrillation and average E prime, which were all significant on univariable analysis. The variables SPAP, TAPSE, and TAPSE/sPAP were added separately to the base model to form models A, B, and C, respectively. **Table (B):** Tests comparing the predictive performance of the three models. . CABG = coronary artery bypass graft; CI = confidence interval; EROA = effective regurgitant orifice area; HR = hazard ratio; LR = likelihood ratio; LV = left ventricle; NRI = net reclassification improvement; NYHA = New York Heart Association; RV = eight ventricle; SPAP = systolic pulmonary artery pressure; TAPSE = tricuspid annular plane systolic excursion, TV = tricuspid valve

of TAPSE/sPAP vs TAPSE or sPAP alone, these variables were added separately to the same base model (**Table 2B**). Adding the TAPSE/sPAP ratio to the base model resulted in a significant net reclassification improvement (NRI: 0.475; 95% CI: 0.102 to 0.846), while TAPSE or sPAP alone did not. Even in a subgroup of patients with only mildly elevated sPAP (30–50 mmHg, n = 355; 71%), RV-PA uncoupling was independently associated with mortality (HR: 2.1; p = 0.028).

Conclusion: In patients with primary MR and elevated pulmonary pressure, RV-PA uncoupling was independently associated with post-surgical mortality, and resulted in better risk stratification of these patients than TAPSE or sPAP alone. TAPSE/sPAP ratio remained independently associated with mortality even in a subgroup of patients with only mildly increased pulmonary pressure. This highlights the importance of assessing RV function in the context of the opposing afterload.

DISTINCT FUNCTIONS OF CARDIAC BETA-ADRENERGIC RECEPTORS IN THE T-TUBULE VS. OUTER SURFACE MEMBRANE

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Beta-adrenoceptors (β-ARs) regulate cardiac function during sympathetic nerve stimulation. They are present in both the cardiac T-tubule (TTM) and outer surface membrane (OSM), but how their location impacts on their function is unknown.

To address this question, we developed a technology based on size exclusion to explore the function of β-ARs located in the OSM. We synthetized a PEG-Iso mole-

cule by covalently linking isoprenaline (Iso) to a 5000 Da PolyEthylene-Glycol (PEG) chain to increase the size of the β-AR agonist and prevent it from accessing the T-tubule network. The affinity of PEG-Iso and Iso on β1- and β2-ARs was measured using radioligand binding. Molecular dynamics simulation was used to assess PEG-Iso conformation and visualise the accessibility of the Iso moiety to water. Using confocal microscopy, we show that PEGylation constrains molecules outside the T-tubule network due to the presence of the extracellular glycocalyx. β-AR activation in OSM with PEG-Iso produced a lower stimulation of [cAMP]i than Iso but a larger stimulation of cytosolic PKA at equivalent levels of [cAMP]I and similar effects on excitation-contraction coupling parameters. However, PEG-Iso produced a much lower stimulation of nuclear cAMP and PKA than Iso.
Thus, OSM β-ARs control mainly cytosolic cAMP/PKA pathway and contractility, while TTM β-ARs control mainly nuclear cAMP, PKA and consequent nuclear protein phosphorylation. Size exclusion strategy using ligand PEGylation provides a unique approach to evaluate the respective contribution of T-tubule vs. outer surface membrane proteins in cardiac cells.

CIRCADIAN DISRUPTION EXACERBATES HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Introduction: Circadian rhythm regulates physiological processes in all living organisms. Disruption of these rhythms, as seen in shift workers, increases the risk for cardiometabolic syndrome (CMS). CMS is a condition characterized by insulin resistance, dyslipidemia, hypertension, and central adiposity. CMS can alter cardiac structure and function which can lead to heart failure with preserved ejection fraction (HFpEF), which disproportionately affects women. While the association between shift work and CMS is well-known, the underlying circadian mechanism of CMS induced HFpEF remains to be poorly understood. This study aims to identify key genes and pathways linking circadian disruption to CMS – induced HFpEF in a sex specific manner.
Methods: C57BL/6J mice were fed high fat diet and L – NAME for 5 weeks to induce CMS induced HFpEF, followed by 5 days of "shift work" protocol achieved by reversing the normal light/dark cycle for 5 days. Body weight, blood pressure and cardiac function via echocardiography were assessed. Hearts were collected for analysis of circadian gene expression (*CLOCK, BMAL1, PER, ROR*) and histological analysis. Blood samples were evaluated for glucose, insulin, lipids, cardiac biomarkers (BNP, NT-proBNP) and inflammatory cytokines.
Results: To validate our model, we assessed diastolic dysfunction and ejection fraction of mice and found that while neither the non-shift work nor shift work CMS mouse models had alterations to ejection fraction, shift work exacerbated diastolic dysfunction in CMS induced HFpEF model. Further, shift work significantly increased blood pressure of CMS mice compared to controls. When examining circadian gene levels, we found that CMS induced HFpEF mouse model had altered circadian gene expression, which was further exacerbated in response to shift work.
Conclusion: This study highlights for the first time the impact of circadian disruption on cardiometabolic dysfunction and its role in HFpEF development. By identifying regulatory genes affected by shift work, this research will support the development of novel therapeutic approaches to treat CMS induced HFpEF in a sex specific manner.

MECHANISMS OF DIAZEPAM-INDUCED VASODILATION IN HUMAN UMBILICAL ARTERIES

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Introduction: Emerging interest in peripheral GABAergic signaling has brought renewed attention to the vascular effects of benzodiazepines, particularly in pregnancy. Although their vasorelaxant actions have been explored in various vascular beds, underlying mechanisms remain poorly defined. This study investigated the pathways mediating diazepam-induced vasodilation in human umbilical arteries.
Methods: Endothelium-intact umbilical artery rings were precontracted with serotonin, and diazepam (0.01–100 μM) concentration–response curves were generated using tissue bath assays. To dissect the mechanism, inhibitors targeting eNOS, COX, potassium and calcium channels, as well as TSPO and 5-HT2A receptors were applied.
Results: Diazepam induced time- and concentration-dependent relaxation of umbilical arteries, achieving nearly 100% effect at 100 μM. This response was independent of endothelial NO or COX pathways. Potassium channel modulators attenuated the effect, supporting channel involvement. Diazepam also reduced $BaCl_2$- and serotonin-induced contractions, suggesting calcium channel inhibition. TSPO blockade with PK11195 significantly reduced vasorelaxation.
Conclusion: Diazepam induces potent, endothelium-independent vasodilation in umbilical artery via potassium channel activation, calcium channel inhibition, and TSPO involvement. These findings underscore diazepam's potential role in modulating umbilical vascular tone, with implications for its use during pregnancy.

CARDIOPROTECTIVE EFFECTS OF NATURAL POLYPHENOLS

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Cardiovascular diseases are among the leading causes of morbidity and mortality worldwide. Unhealthy dietary habits have clearly been shown to contribute to the development of cardiovascular diseases. Beyond the primary nutrients, a healthy diet is also rich in plant-derived compounds. Natural polyphenols, found in fruits, vegetables, and red wine, are considered to improve cardiovascular health. Numerous *in vitro* and *in vivo* pre-clinical studies as well as human clinical trials have shown that the consumption of polyphenol-rich diet, including moderate red wine consumption, may be beneficial in the prevention and treatment of cardiovascular diseases. The primary mechanisms of action for cardioprotective polyphenols include antioxidant, anti-inflammatory processes, and beneficial interaction with various intracellular signalling pathways. In pre-clinical trials, these polyphenols could effectively reduce blood pressure, enhance vascular function, attenuate left ventricular hypertrophy and remodelling while improving left ventricular function. In addition, these polyphenols have potent anti-atherogenic effects and can positively influence several cardiovascular risk factors such as lipid profile, diabetes, and vascular inflammation. A large number of clinical trials have also been performed confirming the potent cardioprotective effects of these polyphenols in humans. Despite of the intensive research, to date, we have only studied a small fraction of the approximately 10,000 described dietary polyphenols leaving a vast area open for further studies.

GENETIC CAUSES OF AORTIC SYNDROMES IN A REPRESENTATIVE CZECH POPULATION SAMPLE

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Background: Aneurysm of great arteries represent a potentially lethal condition due to the risk of dissection, whose assessment is based also on recognition of the molecular genetic mechanism. Our study aimed to evaluate the genetic architecture of aneurysm in a representative cohort.
Methods: In total 1003 patients (626 males and 337 females, mean age 37,5 year) were referred to molecular genetic analysis due the diagnosis of aortic dissection or aneurysm with or without valvular defects. There were 101/1003 individuals who suffered from dissection of aorta, coronary arteries or carotids. Cardiogenetic analysis with next-generation targeted/exom sequencing (SOPHiA GENETICS, Switzerland) was performed.
Results: Genetic cause could be identified in 7,9% of cases (79/1003). In individuals with dissection the genetic yield was 20% (21/101). The most frequently identified P/LP DNA variants in the dissection as well as non-dissection groups were in fibrilin 1 gene (*FBN1*, 46/1003) defining the Marfan syndrome (MFS), followed by genes causing Loeys–Dietz syndrome (*TGFBR1/2, TGFB2, SMAD3*, 18/1003). Other less frequent causes were identified in collagen indicating Ehlers–Danlos syndrome (*COL3A1*, 4/1003, *EDS* IV) or in sarcomeric gene in myosin heavy chain type 11 (*MYH11*, 4/1003). Rare causes were identified in *JAG1, FLNA* or *MFAP5* genes.
Conclusion: Although the genetic stratification in aneurysm may lead to individualized therapy, the genetic yield in those patients is very low. It points out the importance of genetic expertise and family cascade screening in relatives at risk.

ONE-YEAR OUTCOMES OF HIGH-RISK PERCUTANEOUS CORONARY INTERVENTIONS WITH VERSUS WITHOUT MECHANICAL CIRCULATORY SUPPORT: PROPENSITY SCORE-MATCHED ANALYSIS

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Introduction: Impella is increasingly used to provide mechanical circulatory support (MCS) during high-risk percutaneous coronary interventions (HR-PCI). Registry-based data provide insights into the efficacy and safety of Impella use during HR-PCI, until evidence from randomized trials is available.
Aims: We aimed to evaluate one-year outcomes after Impella-assisted HR-PCI compared to HR-PCI without MCS.
Methods: One hundred and thirty-eight patients undergoing Impella-supported PCI included in the IMPELLA-PL registry were propensity score matched in a 1 : 1 ratio with controls who underwent HR-PCI without MCS from the retrospective CardioSilesia registry. Study endpoints included one-year post-discharge all-cause mortality, myocardial infarction (MI), stroke, rehospitalization for heart failure (HF), and repeated coronary revascularization.
Results: Clinical and angiographic characteristics were comparable in both groups, with higher rates of dyslipidemia (78.3 vs. 37.7%, p <0.001), chronic kidney disease (39.9 vs. 16.7%, p <0.001), and peripheral artery disease (34.1 vs. 8.0%, p <0.001) in the IMPELLA-PL cohort. Intravascular ultrasound was more frequently used in the Impella-supported patients (44.2 vs. 25.4%, p = 0.002), while staged revascularization was more common in the control group (30.4 vs. 15.9%, p = 0.007). At one year, rates of all-cause mortality, stroke and repeated coronary revascularization were similar in both groups. MI was less frequent in the IMPELLA-PL cohort (1.4 vs. 10.9%, p = 0.003), along with a trend towards lower incidence of rehospitalizations for HF (9.4 vs. 18.1%, p = 0.055).
Conclusions: Despite unfavorable cardiovascular risk profile, patients from the IMPELLA-PL registry who underwent Impella-assisted HR-PCI had comparable one-year survival, with a lower rate of MI compared to propensity score-matched controls who underwent HR-PCI without MCS. Impella may improve outcomes during HR-PCI, but randomized data are required to confirm this finding.

COMPREHENSIVE CORONARY FUNCTIONAL TESTING IN PATIENTS WITH ANGINA PECTORIS WITH NON-OBSTRUCTIVE CORONARY ARTERIES: A SINGLE-CENTER EXPERIENCE

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Introduction: Angina pectoris with non-obstructive coronary arteries (ANOCA) is a common clinical finding in patients presenting with chest pain. It involves both structural and functional impairment of epicardial arteries and the microcirculation. Two main clinical endotypes are recognized in ANOCA: (1) Microvascular angina (MVA), including coronary microvascular dysfunction (CMD) or microvascular spasm, and (2) vasospastic angina (VSA).
Methods: Comprehensive coronary function testing (CFT) is currently the only diagnostic modality that can definitively establish the ANOCA endotype including coronary angiography, assessment of the hemodynamic significance of stenosis, evaluation of microvascular function using bolus or continuous thermodilution, and Ach provocation testing. Since CFT is not yet routinely performed in the Czech Republic, the aim of our study was to evaluate its feasibility and benefits in everyday clinical practice.
Results: Between August 2024 and July 2025, we implemented a standardized CFT protocol in 31 patients (68% female, median age 66 years; range 22–78 years). CMD was confirmed in 6 patients (19%). Ach testing was positive in 19 patients (61%), 9 with epicardial vasospasm (29%) and 4 with microvascular spasm (13%). Two patients (6%) presented with CMD and microvascular spasm, while four (13%) had CMD combined with epicardial vasospasm. Targeted pharmacological therapy based on CFT findings led to symptom improvement. The mean Seattle Angina Questionnaire (SAQ) score increased from 69.40 at baseline to 79.97 after two months (13.22% improvement). Adverse events were minimal: transient, asymptomatic bradycardia during Ach testing occurred in 6 patients (22.2%), and one patient (3.7%) developed paroxysmal atrial fibrillation, which was promptly resolved with propafenone. All procedures were performed via radial artery access, except one femoral case.
Conclusion: CFT enables accurate endotyping and supports personalized treatment of ANOCA patients. Its implementation into routine clinical practice improves diagnostic accuracy and leads to more effective patient care.

EFFECTS OF POLLUTED AIR ON THE PATHOPHYSIOLOGY OF MYOCARDIAL ISCHEMIA

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Environmental pollution is one of the biggest problems facing the modern world, because it affects the health of virtually all living beings on the planet, increasing morbidity and mortality. We have described the effects of acute and chronic pollution on the lungs and heart, as well as three hypotheses that could explain how polluting particles reach different organs, especially the lungs and heart. Polluting particles reach the lungs through the respiration of living beings, where they activate pro-inflammatory cytokines, which reach the heart, producing damage to the mitochondria that increases the formation of free radicals and decreases the synthesis of ATP. This mechanism leads to a situation of myocardial ischemia whose end point is myocardial infarction. This can occur with or without coronary obstruction, obviously the presence of the latter aggravates the situation. Acute pollution produces cellular and molecular changes that lead to alterations of important cellular structures. Chronic pollution over time produces structural remodeling of the lungs and heart, leading to cardiac pathologies that, due to pressure and volume overload, modify the geometry of the organs. In summary, whether due to acute or chronic exposure to polluting particles, we are in the presence of an important risk factor for cardiovascular health that increases the incidence of myocardial infarction and heart failure in patients exposed to breathing severely polluted air.

HOW RYR2 PHOSPHORYLATION TUNES THE HEART RATE FOR FIGHT OR FLIGHT

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Heart rhythm is initiated and maintained by the automatic activity of sinoatrial node (SAN) cells, which serve as the primary pacemaker of the heart. This automaticity is governed by two interdependent mechanisms often referred to as the "voltage clock" and the "calcium (Ca^{2+}) clock". The ryanodine receptor type 2 (RyR2), the main sarcoplasmic reticulum Ca^{2+} release channel, is a central component of the Ca^{2+} clock.

During acute stress, heart rate increases – a positive chronotropic response – primarily through β-adrenergic receptor (β-AR) stimulation, leading to elevated cAMP levels and activation of protein kinase A (PKA) in SAN cells. While the modulation of the voltage clock by cAMP is well characterized, the possible participation of RyR2 phosphorylation in this context remains less clear.
RyR2 is phosphorylated by PKA at multiple sites, including the well-studied S2808 site located within the phosphorylation hot spot, and the less-characterized S2030 site located in a distinct cytosolic domain. Previous studies have shown that phosphorylation at S2808 is not essential for the SAN response to β-AR stimulation. In this study, we focus on the S2030 site, which may engage different molecular mechanisms and have unique functional implications.
In my presentation, I will share new data investigating the role of RyR2 phosphorylation at S2030 by PKA in regulating SAN automaticity and its participation in the positive chronotropic response during the fight-or-flight reaction. I will also explore potential sex-based differences in this regulatory pathway and discuss the broader implications for arrhythmogenesis under sympathetic stress.

THE COMPARISON OF ECG FINDINGS IN PATIENTS WITH LIGHT-CHAIN AND WILD-TYPE TRANSTHYRETIN CARDIAC AMYLOIDOSIS AT THE TIME OF DIAGNOSIS

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Background and aim of the study: The two most common types of cardiac amyloidosis (CA) are light-chain (AL) and transthyretin (ATTR) amyloidosis. ECG represents a basic diagnostic tool in the initial evaluation of CA. Little is known about the differences in ECG characteristics between both types of CA. Therefore, the aim of our study was to compare ECG findings in newly diagnosed patients with AL and wild-type ATTR CA, respectively.
Methods: We retrospectively evaluated ECG data in a cohort of newly diagnosed patients with AL (n = 50, years 2010–2024) and wild-type ATTR CA (n = 50, years 2023–2024). In all cases, the diagnosis of CA was made according to current recommendations. Data are expressed as mean ± SD or as a number and/or percentage of subjects.
Results: The mean age of patients with AL CA was 67 ± 10 years (72% males) and 77 ± 7 years in wild-type ATTR individuals (88% males) (p <0.01 for age; p = 0.04 for gender). Atrial fibrillation, normal QRS voltage (for both p <0.01), permanently paced rhythm, as well as left anterior fascicular block alone, or either in combination with first-degree AV block or right bundle branch block (for all

p <0.05) were more frequently present in ATTR patients. Low QRS voltage was found more frequently in AL subjects (p <0.01), while ECG pattern consistent with left ventricular hypertrophy was present only in one patient with AL CA an in none ATTR individual.
Conclusions: In both types of CA, a striking finding is the almost complete absence of ECG criteria of left ventricular hypertrophy, contrasting with the increased left ventricular wall thickness, which is well-known "red-flag" feature for CA. Compared to AL, left anterior fascicular block, either alone or in combination with first-degree AV block or right bundle branch block (complete or incomplete), atrial fibrillation and permanently paced rhythm are significantly more common in wild-type ATTR CA. On the other hand, low QRS voltage is more frequently present in AL CA. Although these ECG characteristics cannot reliably differentiate between both diseases, they may be helpful in the initial diagnostic work-up.

COMPARISON OF PROCEDURAL WORKFLOW, SAFETY, AND ONE-YEAR EFFICACY OF CATHETER ABLATION OF ATRIAL FIBRILLATION USING RADIOFREQUENCY AND PULSED-FIELD ENERGY

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Introduction: Pulsed-field (PF) ablation is an emerging technology in catheter ablation of atrial fibrillation (AF). This study sought to show a single-center comparison of procedural workflow in AF ablation using PF and radiofrequency (RF) energy.
Methods: This is a single center, non-randomized, cohort, observational study. All consecutive patients addmited for AF ablation were enrolled. Ablation was performed using PF energy by pentaspline catheter (Farapulse, BSCI, USA), or by RF energy (SmartTouch or QDot, Biosense-Webster, USA). In paroxysmal patients, only PVI was done in all patients. In non-paroxysmal patients, also posterior wall and mitral isthmus ablation were performed in all patients in the PFA group. In the RF group, additional ablations beyond PVI were left on the discretion of the operator. Cavotricuspid isthmus ablation was performed in patients with documented typical atrial flutter. All patients underwent 24-hour Holter monitoring and clinical check-up 3, 6, 9 and 12 months after AF ablation to assess SR maintenance.
Results: A total of 159 patients were enrolled, 95 in the PFA and 61 in the RFA group. Despite non-randomized design, both groups were similar with respect from the baseline clinical characteristics (PFA vs. RFA): age 63.1 + 11.3 vs. 64.5 + 10.9 years, BMI 29.7 + 5.2 vs. 30.2 + 5.1 kg/m^2, 62 (65.3%) vs. 44 (72.1%) men, 51(53.7%) vs. 29 (47.5%) paroxysmal AF, 29 (30.5%) vs. 13 (21.3%) with history of heart failure, left ventricular ejection fraction 55.5% vs. 55.4% (all p = n.s.). Mean procedural time was substantially shorter with PFA (60.2 + 18.6 vs. 144.9 + 61.2 min, p <0.001), as well as LA dwelling time (38.18 + 9.5 vs. 121.4 + 58.9 min, p <0.001). One major adverse event occurred in each group (cardiac arrest with a need for resuscitation and mechanical ventilation in the PFA, and one pericardial effusion in the RF group; 0.6 vs. 1.1%). AF freedom without any arrhythmia recurrence after 12 months follow up was present in 74.4% vs. 73.7 % patients.
Conclusion: Pulsed-field ablation using pentaspline catheter is asssociated with significantly shorter procedural time, similar safety and similar one-year efficacy, as compared to RF ablation.

ASSOCIATION BETWEEN BODY MASS INDEX, LEFT VENTRICULAR REMODELING, AND LONG-TERM OUTCOME IN PATIENTS WITH BICUSPID AORTIC VALVE

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Introduction: The obesity paradox, i.e. higher body mass index (BMI) being associated with improved outcome, has never been examined in patients with bicuspid aortic valve (BAV).

Table 1 – Baseline and echocardiographic characteristics of three groups of BMI in bicuspid aortic valve

	Overall (n = 4161)	Normal (n = 1751)	Overweight (n = 1598)	Obese (n = 812)	*p*
Age (mean [SD]), year	49.30 (16.61)	46.48 (18.13)	51.22 (15.52)*	51.57 (14.24)*	<0.001
Female	1224 (29.4)	580 (33.1)	382 (23.9)*	262 (32.3)†	<0.001
BSA (mean [SD]), m^2	1.93 (0.24)	1.78 (0.18)	1.97 (0.18)*	2.16 (o.24)*,‡	<0.001
BMI (mean [SD]), kg/m^2	26.48 (4.95)	22.35 (1.97)	27.19 (1.40)*	33.99 (4.39)*,†	<0.001
Hypertension (%)	951 (24.0)	291 (17.5)	407 (26.8)*	253 (32.4)*,‡	<0.001
Dyslipidemia (%)	660 (16.3)	189 (11.1)	295 (19.0)*	176 (21.9)*	<0.001
Diabetes (%)	353 (9.0)	99 (6.0)	132 (8.8)*	122 (15.7)*,‡	<0.001
Smoking (%)	559 (14.2)	236 (14.3)	216 (14.4)	107 (13.8)	0.929
Aortopathy[a] (%)	1193 (31.4)	415 (26.0)	501 (34.5)*	277 (36.9)*	<0.001
LV mass (mean [SD]), g	216.12 (91.49)	199.05 (91.02)	224.14 (86.90)*	238.71 (94.76)*,‡	<0.001
LVMi (mean [SD]), g/m^2	111.23 (43.38)	110.48 (46.25)	113.00 (40.83)	109.29 (41.63)	0.207
RWT (mean [SD])	0.42 (0.11)	0.41 (0.11)	0.43 (0.11)*	0.43 (0.10)*	<0.001
LVEF (mean [SD]), %	59.20 (9.89)	59.76 (9.75)	59.30 (9.97)	57.73 (9.90)*,†	<0.001
Significant AR (%)	951 (23.8)	442 (26.3)	360 (23.6)	149 (19.0)*,‡	<0.001
Significant AS (%)	1261 (31.6)	496 (29.5)	493 (32.3)	272 (34.7)*	0.028
LV remodeling pattern (%)					<0.001
Normal geometry	1008 (36.5)	487 (41.2)	346 (32.6)	175 (33.9)	
Concentric remodeling	552 (20.0)	189 (16.0)	235 (22.1)	128 (24.8)	
Eccentric hypertrophy	527 (19.1)	241 (20.4)	209 (19.7)	77 (14.9)	
Concentric hypertrophy	673 (24.4)	266 (22.5)	271 (25.5)	136 (26.4)	

* *p* <0.05 vs. group normal. † p < 0.05 vs. group overweight. [a] Aortopathy defined as ascending aorta diameter >40 mm. AR – aortic stenosis; BMI – body mass index; BSA – body surface area; LVEF – left ventricular ejection fraction; LVMi – left ventricular mass index; RWT – relative wall thickness.

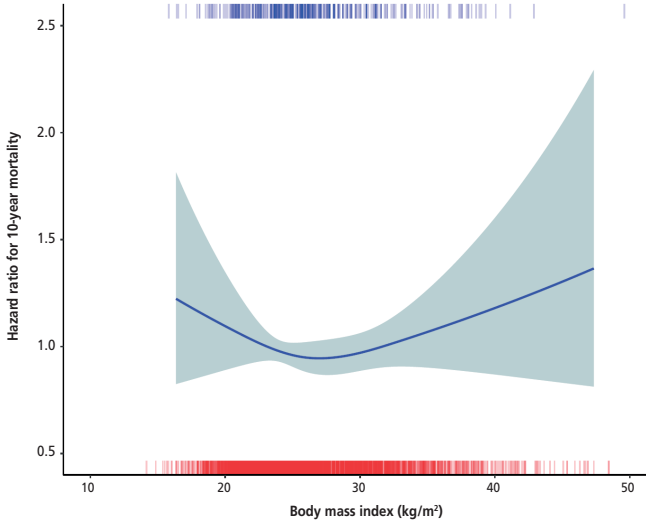

Fig. 1 – Restricted cubic spline between body mass index and 10-year mortality.

Purpose: To explore the association between BMI and outcome and the possible influence of left ventricular (LV) remodeling in a large cohort of BAV patients.
Methods: adult patients diagnosed with BAV from a multicenter registry were included. Patients were stratified into normal weight (<25 kg/m^2), overweight (≥25 and <30 kg/m^2), and obesity (≥30 kg/m^2). Clinical and echocardiographic characteristics were collected, and LV hypertrophy was defined as a LV mass index of >115 g/m^2 in males and > 95 g/m^2 in females. LV remodeling was then further classified as: (i) normal geometry (no LV hypertrophy and relative wall thickness [RWT] ≤0.42); (ii) concentric remodeling: no LV hypertrophy, RWT >0.42; (iii) concentric hypertrophy: LV hypertrophy, RWT >0.42; and (iv) eccentric hypertrophy: LV hypertrophy, RWT ≤0.42. The primary outcome was 10-year all-cause mortality. Aortic valve replacement during follow up was coded as a time-dependent covariate.
Results: A total of 4533 patients (mean age 49 ± 17 years, 29% female) were included. The normal weight, overweight, and obesity group had 1751, 1598, and 812 patients, respectively. These 3 groups showed significant clinical and echocardiographic differences (**Table 1**). In particular, obese patients had the lowest prevalence of significant aortic regurgitation (overall *p* <0.001), and the highest frequency of significant aortic stenosis (overall *p* = 0.028). Also, LV remodeled significantly differently across the BMI spectrum (overall *p* <0.001), where the obese patients demonstrated the lowest proportion of eccentric hypertrophy but the highest frequency of concentric remodeling. During a median follow up of 2019 (769, 3600) days, 349 events occurred. Overweight patients showed the best survival, demonstrated by the U-shaped restricted cubic spline curve (**Fig. 1**). In the multivariable analysis adjusting for age, sex, comorbidities, LV ejection fraction, significant aortic valve disease, aortopathy, and surgery, both the BMI category (overweight as reference, normal weight: HR 1.44, 95%CI 1.04–2.00, *p* = 0.027; obesity: HR 1.63, 95% CI 1.11–2.39, *p* = 0.012) and LV remodeling patterns (concentric remodeling: HR 1.87, 95% CI 1.17–2.97, *p* = 0.008; concentric hypertrophy: HR 1.77, 95% CI 1.15–2.73, *p* = 0.009; eccentric hypertrophy: HR 1.04, 95% CI 0.62–1.73, *p* = 0.89) were independently associated with 10-year mortality.
Conclusions: In BAV patients, BMI is associated with different extents of LV hypertrophy. Obese patients

are more likely to present with concentric remodeling and less likely with eccentric hypertrophy. The association between BMI and mortality appeared to be "U-shaped", with overweight patients having the most favorable survival even after adjusting for important covariates.

THE VAGO-SPLENIC AXIS IN CARDIOPROTECTION

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A cardioprotective role of electrical stimulation of the vagal nerves is well established. Such electrical vagal nerve stimulation reduces infarct size from coronary occlusion and reperfusion as well as arrhythmias in various animal species. Transcutaneous electrical stimulation at the auricular tragus also activates vagal nerves and reduces infarct size and arrhythmias in patients with acute ST segment elevation infarction (JACC CV Interv 2017;10:1521–1522). Remote ischemic conditioning by brief occlusion and reperfusion of the limbs or peripheral tissue also reduces infarct size in various animal species. In pigs with 60 min occlusion of the distal LAD and 180 min reperfusion infarct size is reduced by remote ischemic conditioning by 4 cycles of 5 min limb occlusion and 5 min reperfusion before (pre-) and during (per-conditioning) during coronary occlusion. Protection by remote ischemic preconditioning is abrogated by bilateral cervical vagotomy, evidencing a mandatory signal transduction through vagal nerves. The protection is also abrogated by splenectomy or by splenic denervation, demonstrating a decisive role of the spleen in the cardioprotection by remote ischemic conditioning. Conversely, stimulation of isolated perfused rat spleens by the vagomimetic carbachol makes them release cardioprotective factor(s) which reduce infarct size in isolated perfused rat hearts with global ischemia/reperfusion (Circ Res 2018;123:1152–1163). In healthy human volunteers both, transcutaneous electrical stimulation at the auricular tragus and remote ischemic conditioning by 3 cycles of 5 min forearm occlusion/5 min reperfusion induces the release of cardioprotective factor(s) into the circulation, since a plasma dialysate from a venous blood sample reduces infarct size in isolated perfused rat hearts with 30 min global ischemia/120 min reperfusion. Such cardioprotection is not seen in healthy volunteers with prior splenectomy (Eur Heart J 2024;45:3164–3177). Our data provide evidence for a mandatory role of vagal activation in cardioprotection by remote ischemic conditioning and the spleen as a decisive relay organ which releases soluble cardioprotective factor(s) upon vagal activation (Nat Rev Cardiol 2025;7:497–509).

CARDIOLOGY ON HIGH BEAM: OBSTACLES AND OPPORTUNITIES

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Introduction: In many parts of the world, the acutely lethal, atherothrombotic manifestations of cardiovascular diseases are being replaced by chronic manifestations, *viz.* heart failure.
Methodology: Yet, despite these impressive successes, new challenges have emerged stemming from the global syndemic of cardiometabolic diseases, challenges associated with cancer survivorship, and more. We have witnessed impressive victories, and yet new challenges have arisen.
Results and conclusion: At the same time, we are now equipped with an impressive array of new approaches to deal with these challenges. Evolving obstacles are met with powerful new weapons, heralding an exciting future. Indeed, I submit that there has never been a better time to be involved with cardiovascular medicine and science.

ROLE OF PROTEIN KINASE A AND CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II IN BETA-ADRENERGIC REGULATION OF POTASSIUM CHANNELS

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Introduction: Acute β-adrenergic receptor (β-AR) stimulation robustly regulates cardiac potassium currents to shorten the ventricular action potential (AP). Besides the protein kinase A pathway, downstream effects are also mediated by calcium/calmodulin-dependent protein kinase II (CaMKII). Our aim was to investigate the involvement of CaMKII in mediating β-AR activation on the most important potassium currents flowing during canine ventricular AP.
Methods: Experiments were carried out on isolated cardiomyocytes originating from canine left ventricles. Inward rectifier potassium current (IK1-50 μM $BaCl_2$), rapid (IKr-1 μM E-4031) and slow delayed rectifier potassium current (IKs-1 μM HMR-1556) were measured under action potential voltage-clamp and IK1 also under conventional voltage-clamp conditions. Data were collected in six study groups: [1] Control (CTRL), [2] β-AR stimulation-with 10 nM isoproterenol (ISO), [3] CaMKII inhibition (1

μM KN-93), [4] PKA inhibition (3 μM H-89), [5] KN-93+ISO, [6] H-89+ISO.
Results: IKr was not different significantly between the groups studied. Reducing intracellular Ca^{2+} concentration with 1 μM nisoldipine decreased the peak and mid-plateau densities of IKs, reduced the current integral. ß-AR activation by ISO resulted in larger IKs densities and integral, and shorter time-to-peak value. These effects of isoproterenol on IKs were significantly smaller when the CaMKII inhibitor 1 μM KN-93 was present in the cells, but the PKA inhibitor 3 μM H-89 did not exert such effect. All effects of isoproterenol on IKs have fully developed even in the presence of 1 μM nisoldipine.
Acute application of 10 nM ISO significantly increased IK1 under the plateau phase of the action potential (0 ± 20 mV) using APVC, and similar results were obtained with conventional voltage clamp. However, β-AR stimulation did not affect the peak current density flowing during terminal repolarization or the overall IK1 integral. The ISO-induced enhancement of IK1 was blocked by the CaMKII inhibitor KN-93 but not by the protein kinase A inhibitor H-89. Neither KN-93 nor H-89 affected the IK1 density under baseline conditions (in the absence of ISO).
Conclusion: CaMKII activation plays an important role in beta-adrenergic stimulation of potassium currents. Beta-adrenergic enhancement of IKs is partly, whereas enhancement of IK1 is mainly mediated by CaMKII activation.

LIRAGLUTIDE RESTORES ZINC HOMEOSTASIS AND MITOCHONDRIAL FUNCTION IN AN INSULIN-RESISTANT SENESCENT CARDIOMYOCYTE MODEL

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Background and aim: Insulin resistance and ageing contribute to cardiomyocyte dysfunction by impairing mitochondrial function, increasing oxidative stress, and disrupting metal ion homeostasis. GLP-1 receptor agonists like liraglutide provide cardioprotective effects beyond glycemic control, including improved mitochondrial performance and antioxidant defence. Zinc is vital for insulin signalling and antioxidant systems, and its imbalance worsens cardiac vulnerability. Whether GLP-1R activation restores zinc handling in insulin-resistant ageing cardiomyocytes remains unclear. This study examined liraglutide's effects on intracellular zinc, mitochondrial integrity, oxidative stress, and ER stress in an insulin-resistant senescent cardiomyocyte model.
Materials and methods: Human AC16 cardiomyocytes were treated with palmitic acid (50 μM) and D-galactose (50 mg/mL) for 24 h to induce insulin resistance and senescence. Cells were exposed to liraglutide (1 μM) acutely (30 min) or chronically (6 h). ROS (DCFH-DA), mitochondrial membrane potential (JC-1), and intracellular zinc (FluoZin-3) were measured by confocal microscopy. ER stress and mitophagy markers were assessed by qPCR and immunoblotting.
Results: Palmitic acid and D-galactose induced insulin resistance and senescence. Chronic liraglutide lowered ROS, restored mitochondrial membrane potential, and increased intracellular zinc ~5-fold. Liraglutide also modulated ER stress and supported mitochondrial quality control.
Conclusion: Sustained liraglutide improved mitochondrial function, redox balance, and zinc homeostasis in insulin-resistant senescent cardiomyocytes, highlighting its potential benefit in age-related metabolic cardiac dysfunction.

MOLECULAR ADAPTATIONS IN THE HEART FOLLOWING PERINATAL HYPOXIA: INSIGHTS FROM MULTI-OMICS ANALYSIS

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Introduction: Cardiovascular diseases are the leading cause of death and disability worldwide. Adverse fetal and perinatal conditions, such as hypoxia, have been strongly linked to increased cardiovascular risk in adulthood. However, the mechanisms behind this link remain unclear. This study explores the long-term cardiac effects of perinatal hypoxia using a multi-omic approach, with a focus on sex differences.
Methods: Pregnant Wistar rats were randomly divided into normoxic or hypoxic groups. The hypoxic group was exposed to chronic continuous hypoxia (FIO_2 0.12) from gestational day 16 until birth. After delivery, all animals were raised in normoxic conditions. Hearts were collected from male and female offspring at postnatal days (P) 1, 4, 7, 28, and 90. Left ventricular structure and function were assessed via echocardiography at P28 and P90. RNA sequencing was used for transcriptomic analysis, data-independent acquisition mass spectrometry for proteomics, and a multiplatform LC-MS approach for metabolomics and lipidomics at P1, P4, P7, P28, and P90.
Results: Echocardiography revealed mild concentric hypertrophy in both sexes at P28. At P90, hypoxic females showed increased relative wall thickness, while only minor functional changes were observed, including higher

heart rate at P28 and reduced pulmonary artery flow in hypoxic males. These findings suggest that perinatal hypoxia has a more pronounced impact on male hearts. Regarding transcriptomics, the most notable effect of perinatal hypoxia was observed at P1, regardless of sex. Proteomic and metabolomic profiling revealed the most significant changes at P4 between normoxic and hypoxic males and females, while lipidomic analysis showed the greatest differences at P28. These changes were more pronounced in males.

Conclusion: This study reveals that perinatal hypoxia induces a long-term impact on myocardial molecular profiles, highlighting notable sex differences. These findings emphasize the importance of considering sex differences in the developmental origins of cardiovascular disease and may help identify potential targets for early interventions.

ELECTROPHYSIOLOGICAL REMODELLING OF THE PULMONARY VEIN IN A CANINE MODEL OF TRAINING-INDUCED ATRIAL FIBRILLATION

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Introduction: The risk of atrial fibrillation (AF) is known to be elevated by long-term endurance exercise, though the underlying pathophysiological mechanisms are poorly understood. In this study, we examined whether electrophysiological remodelling of the pulmonary vein (PV) myocardium contributes to this elevated AF risk, using a translational (canine) model of high-intensity endurance exercise.

Methods and results: Adult Beagle dogs were randomised into sedentary (Sed, n = 14) and trained (ExT, n = 14) groups. ExT-dogs underwent treadmill training for 16 weeks (5 days/week, 280 min/day). At the end of the training period, epicardial stimulation, burst pacing, and concomitant PV monophasic action potential (MAP) recordings were performed in open-chest animals. Following an intravenous bolus of acetylcholine (5μg/kg), ExT dogs exhibited a significantly higher susceptibility to pacing-induced AF (incidence: Sed, 13.6 ± 4.3%; ExT, 42.8 ± 7.5%; duration: Sed, 2.2 ± 0.8 s; ExT, 8.3 ± 1.9 s; all p <0.05). A slightly shortened left atrial (LA) MAP duration was also observed in the ExT group (APD_{90}: Sed, 159.2 ± 14.1 ms; ExT, 115.2 ± 8.4 ms; p <0.05). Multielectrode array mapping at the LA-PV junction demonstrated significantly altered radial stimulus propagation in ExT dogs (mean propagation direction: Sed, 96 ± 14°; ExT, 162 ± 24°; p <0.05). In ex vivo LA-PV preparations, spontaneous AP generation was observed in 83.3% of ExT PVs in the presence of 1 μM isoproterenol, compared to 0% in Sed PVs (p <0.05). Following 10 Hz pacing combined with 1 μM isoproterenol exposure, the incidence of self-sustaining evoked APs was significantly higher in ExT than in Sed (Sed, 23%; ExT, 83%; p <0.05). These APs lasted significantly longer in ExT animals (ExT, 39.6 ± 21.3 s; Sed, 0.3 ± 0.3 s; p <0.05) and occurred at a higher frequency (ExT, 111.8 ± 40.5 beats/min; Sed, 10.3 ± 10.3 beats/min; p <0.05). Data are expressed as mean ± SEM.

Conclusions: This is the first demonstration of training-induced electrophysiological remodelling of the pulmonary veins in a large animal model that provides translational insight into the electro-anatomical PV-substrate predisposing athletes to AF.

SEX DIFFERENCES IN CARDIAC DAMAGE DUE TO PRESSURE OVERLOAD: THE ROLE OF INFLAMMATION AND OXIDATIVE STRESS

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Introduction: Pressure overload is a primary driver of cardiac damage, characterised by hypertrophy, fibrosis, and progressive decline in cardiac function. However, the precise molecular mechanisms which accelerate the harmful conditions compromising heart morphology and contractility are yet to be fully described.

Methodology: Abdominal aortic constriction (AAC), induced at day 2 post-birth, was used in female and male rats. Heart damage due to progressive pressure overload, developing until day 90, was assessed echocardiographically, and comprehensive molecular and histological analyses of the left ventricles were performed to assess the underlying mechanisms.
Results: The ratios of left and right ventricle to body weight (BW) were increased in both sexes, while the lung/BW ratio was increased in male AAC rats only. Both sexes showed a decrease in fractional shortening and ejection fraction; however, male AAC rats had also increased LV diameter in systole and decreased heart rate compared to the respective control group. The collagen content, along with pro-oxidative enzymes such as NOX2, NOX4 and iNOS, was increased in male, but not in female AAC rats. Contrary, pro-inflammatory cytokines such as TNF and IL-6 and IFN-γ were upregulated in female AAC rats only. In spite of these sex-dependent mechanisms, programmed necrosis, assessed as the elevated levels of pRIP3 and pMLKL, was activated in both female and male AAC rats.
Conclusion: Male AAC rats faced a rather pro-oxidative environment, while female AAC hearts showed a more targeted pro-inflammatory damage, both of which might underlie programmed necrosis-like cell loss. Thus, our findings have advanced the current understanding of sex differences in pressure overload-induced cardiac injury and advocated for sex-dependent personalised cardioprotective strategies.

SEX DIFFERENCES IN CARDIAC REMODELING INDUCED BY EARLY ABDOMINAL AORTIC CONSTRICTION IN WISTAR RATS

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Left ventricular pressure overload (LVPO) in adults is associated with adverse electrical remodeling, characterized by reduced conduction velocity (CV) and an increased risk of arrhythmias. However, the progression of LVPO differs when imposed during the proliferative phase of cardiac development. It remains unknown how increased cardiomyocyte proliferation affects cardiac remodeling following LVPO and whether there are sexual differences in this process.
This study aimed to characterize left ventricular (LV) geometry and function in Wistar rats with increased pressure load induced by abdominal aortic constriction (AAC) in the early (proliferative) postnatal period and to determine the sex differences.
In newborn pups (postnatal day 2), surgical AAC induction was performed by ligating the aorta in the supradiaphragmatic suprarenal region using a 0.25 mm internal diameter ligature. In control groups, the aorta was exposed but not constricted. Cardiac function and geometry were assessed by echocardiography. Cardiac electrophysiology was assessed by optical mapping, and myocardial fibrosis was assessed by immunohistochemistry.
At postnatal day 90, AAC resulted in LV dilatation (LVDd: 9.26 ± 0.23 mm vs. 8.10 ± 0.12 mm and 8.15 ± 0.27 mm vs. 7.15 ± 0.09 mm) and LV wall thickening in both males and females compared to controls. Relative LV mass expressed as LVW/BW was similar in males and females (LVW/BW ratio increased by 103 ± 9% and 99 ± 10% compared to controls). AAC also resulted in a deterioration of systolic function in both males and females (FS: 30.3 ± 1.4% vs. 41.6 ± 1.0% and 33.4 ± 1.8% vs. 43.2 ± 0.9%) and a decrease in heart rate. However, cardiac output was unchanged in both sexes. Relative right ventricular and lung weights were increased in both sexes by AAC, with a greater increase in males. AAC-induced cardiac remodeling was further associated with a higher rate of myocardial fibrosis in males than in females and a decrease in conduction velocity (CV) in males compared to controls (CVL 80 ± 3 vs. 92 ± 3 cm/s and CVT 35 ± 2 vs. 54 ± 3 cm/s), but not in females (CVL 104 ± 8 vs. 109 ± 8 cm/s and CVT 66 ± 7 vs. 59 ± 6 cm/s). Our data show that early postnatal left ventricular pressure overload induced by AAC leads to similar changes in cardiac geometry in male and female Wistar rats. This cardiac remodeling exhibits sex differences, leading to proarrhythmogenic changes in males but not females.

INTERMITTENT FASTING AND MYOCARDIAL ISCHEMIA-REPERFUSION INJURY IN MICE: EFFECT OF HIF-1α

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Intermittent fasting (IF) is a promising dietary approach associated with a range of health benefits. Characterised by alternating periods of eating and fasting, IF has demonstrated potential in mitigating the risk of metabolic disorders and cardiovascular diseases. Despite these benefits, the precise molecular mechanisms that confer cardiac protection remain incompletely understood. In this context, hypoxia-inducible factor (HIF-1α) emerges as a potential mediator, responsive to both oxygen and nutrient availability. Additionally, cardiovascular diseases have also been linked to RNA modifications, particularly the epitranscriptomic mark known as N6-methyladenosine (m6A), which can modulate HIF-1α activity and influence myocardial tolerance to ischemia-reperfusion injury.

To investigate the interplay between IF, HIF-1α, and m6A RNA modification in cardiac protection, adult male wild-type (wt) mice and HIF-1α heterozygous knockout (Hif1a+/–) mice were divided into four experimental groups: ad libitum feeding or alternate-day fasting for 8 weeks. After this period, ischemia-reperfusion injury was induced ex vivo using the Langendorff technique. We also examined the impact of IF on the gene expression of key m6A regulators by the qPCR method.
The findings showed that IF significantly reduced the myocardial infarct size and was associated with elevated blood ketone levels and decreased glucose levels. However, no differences were observed between Hif1a± and wt mice.
In conclusion, our results support the protective potential of IF against acute myocardial infarction. Nevertheless, we showed that partial deletion of HIF-1α did not affect myocardial infarct size, ketone body levels, or the expression of m6A regulatory genes in mice.

IMPACT OF LOSARTAN ON ANGIOTENSIN II-INDUCED CARDIAC FIBROSIS AND ROLE OF SCLERAXIS IN THIS PATHWAY

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Theme: Heart failure and cardiomyopathy

Introduction: Cardiac fibrosis accelerates heart failure and death, and despite its impact on millions of people worldwide, it currently has no treatment options. Angiotensin II (AngII) contributes to cardiac fibrosis, and its inhibitor losartan, despite showing anti-fibrotic effects, has not been accepted as a treatment option as its mechanism in cardiac fibrosis is unclear. AngII pathway activation induces nuclear translocation of myocardin-related transcription factor-a (MRTF-A) which along with serum response factor (SRF) accelerates transition of fibroblasts to myofibroblasts, a process regulated by the transcription factor scleraxis. Scleraxis transcriptionally regulates pro-fibrotic gene expression, and its deletion can prevent and reverse pressure overload-induced cardiac fibrosis.
Hypothesis- AngII works through an MRTF-A/SRF/scleraxis pathway to induce cardiac fibrosis and anti- fibrotic effects of losartan are linked to scleraxis function.
Results: AngII delivery significantly increased blood pressure and cardiac hypertrophy compared to the saline control group, which was significantly reduced by losartan treatment. Losartan significantly attenuated the enhanced expression of pro-fibrotic genes and scleraxis in the AngII group, indicating involvement of scleraxis in this fibrosis pathway. In vitro results in rat cardiac fibroblasts showed that AngII treatment enhanced scleraxis expression and scleraxis knockdown showed that scleraxis is required for AngII to have its maximal impact on collagen expression levels. MRTF-A and SRF regulated scleraxis expression by binding to and transactivating the scleraxis promoter across species.
Conclusions: AngII activates an MRTF-A/SRF/scleraxis pathway, and both losartan and targeted downregulation of scleraxis can be effective strategies against cardiac fibrosis.

HIPSC-CM AS A MODEL TO STUDY EXERCISE-INDUCED ARRHYTHMIAS IN HCM?

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Hypertrophic cardiomyopathy (HCM) is a classic genetic heart disease. Therefore, human pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) should reflect the typical (patho)physiological abnormalities on a human electrophysiological background. In principle, using hiPSC-CM, it should be possible to study pathophysiological mechanisms. We have used 3dimensional model of engineered heart tissues (EHT) from hiPSC-CM lines bearing different HCM mutations and from respective isogenic controls. We measured action potentials (AP) with sharp microelectrodes in free-running EHT. Catecholamines, rise in extracellular potassium, drop in pH and high temperature was used to mimic the effect of exercise. While control EHT tolerated all four interventions, even when applied at the same time, hiPSCM-EHT showed substantial beating irregularity and afterdepolarizations even with a single intervention. We feel confident that such an approach could help to identify arrhythmia mechanisms in different genetic backgrounds of HCM. Furthermore, it should allow identification of promising drug treatment strategies.

AN INVESTIGATION ON THE EFFECTS OF A PHENOLIC-CONTAINING COMPOUND PINE BARK EXTRACT IN AN AGING-INDUCED AC16 CELLS

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Aging is a critical risk factor for cardiovascular diseases, often associated with mitochondrial dysfunction and structural deterioration in cardiomyocytes. This study aimed to evaluate the functional effects of a phenolic-rich pine bark extract on aging-induced human cardiomyocyte-like AC16 cells, with a specific focus on mitochondrial integrity and bioenergetics. AC16 cells were cultured under specific conditions to maintain their proliferative capacity and cardiomyocyte-like properties. Insulin-resistant cellular senescence was induced by a 24-hour incubation with D-galactose (50 mg/mL) and palmitic acid (50 µM) and then confirmed by β-galactosidase staining. Following induction of senesence, cells were incubated with the supplement (500 µM) under either acute or chronic conditions. Mitochondrial function was evaluated by assays measuring membrane potential (MMP) and reactive oxygen species (ROS) levels. Fluorescence examinations revealed improved MMP in the insulin-resistant senescence model under chronic supplement incubation. Interestingly, ROS levels were reduced only during the acute supplement application. Biochemical analysis in senescence cells, such as pyrin domain-containing protein 3, NF-κB-P65, nuclear respiratory factor 2, Drp1, 4-hydroxynonenal, and Bax/Bcl-2, could strongly support our electrophysiological results in mitochondrial energetics. Our findings demonstrate that pine bark extract significantly improves mitochondrial membrane potential, enhances ATP synthesis, and reduces oxidative stress markers in aging-induced cells. These results suggest that phenolic compounds from pine bark may exert cardioprotective effects through mitochondrial stabilization, highlighting their potential as therapeutic agents in age-related cardiac dysfunction.

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CARDIAC INVOLVEMENT IN TAKAYASU ARTERITIS: A CASE OF CONCURRENT CORONARY ARTERY DISEASE AND SEVERE AORTIC REGURGITATION

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Background: Takayasu arteritis is a rare, chronic granulomatous vasculitis involving large and medium-sized arteries, particularly the aorta and its major branches. The disease is characterized by mononuclear infiltration and granulomatous inflammation of the vascular media, resulting in arterial wall thickening, stenosis, occlusion, or aneurysmal dilation. While vascular involvement predominates, cardiac manifestations such as aortic regurgitation and coronary artery disease may significantly affect prognosis.
Case summary: We report the case of a 54-year-old woman with a history of ankylosing spondylitis and thrombophilia, presenting with typical anginal chest pain, exertional inspiratory dyspnea, and fatigue. Clinical examination revealed a diastolic murmur at the left third intercostal space and absent peripheral pulses over the left axillary and brachial arteries. Transthoracic echocardiography showed a dilated left ventricle with preserved systolic function and thickened aortic valve leaflets with restricted motion, resulting in a severe aortic regurgitation. Thoracic CT angiography demonstrated a normal origin of the right coronary artery and absence of the left main coronary artery, with separate ostial origins of the LAD and circumflex arteries, both with critical ostial stenoses, later confirmed by invasive angiography. The thoracic aorta was of normal caliber but showed diffuse concentric wall thickening from the annulus to the isthmus, involving the coronary ostia – features consistent with large-vessel vasculitis. A high-grade stenosis (60–70%) was also found in the left subclavian and axillary arteries, with occlusion of the left internal mammary artery. These findings confirmed Takayasu arteritis with extensive aortic and coronary involvement. The patient underwent combined surgical treatment consisting of double aorto-coronary bypass and a Bentall procedure.
Conclusion: The case highlights the challenges of diagnosing and managing Takayasu arteritis with combined valvular and coronary disease. In the absence of conventional risk factors, ostial coronary stenoses and aortic regurgitation should prompt consideration of large-vessel vasculitis.

THE EFFECTS OF IMIQUIMOD INDUCED PSORIASIS LIKE PATHOLOGY ON CARDIOVASCULAR FUNCTION AND REDOX BALANCE

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Psoriasis represents complex disorder with multiple etiological factors and pathogenetic mechanisms which affect a large number of organ systems in addition to the skin. Patients suffering from psoriasis are in a higher risk of cardiovascular disease, including heart attack and stroke. This association is thought to be due to shared inflammatory pathways, oxidative damage, and risk factors between the two conditions. Patients with severe psoriasis are at a greater risk of CVD, and their risk can be two to three times higher than in those without psoriasis. The aim of this study was to compare the changes in skin and cardiovascular function between two immunogenetically different strains of rats, Dark Agouti (DA) – predominantly Th1 – and Albino Oxford (AO) – predominantly Th2. For seven consecutive days, a 50 mg dose of 5% imiquimod cream was topically applied to the shaved back skin of the rats in the psoriasis groups on a daily basis. After the experimental protocol was completed, the animals were sacrificed, and the skin samples on which psoriasis was induced were histomorphologically analysed. Cardiac function was examined *in vivo*, echocardiographically, and *ex vivo*, using the Langendorff technique of isolated mammalian hearts. Results of the study showed the physiological structure of the skin in both groups of healthy animals, with hair follicles and sebaceous glands, the stroma of the dermis is filled with fibers and fibroblasts. More prominent changes were found in AO rats with psoriasis compared to DA rats. Parakeratosis, hypergranulosis, and spongiosis of the epidermis are present. Epidermal layers are flattened, and inflammatory cells permeate the epidermis, suprabasal vesicles filled with acantholytic cells, neutrophils, lymphocytes, and histiocytes are observed. In line with the skin changes, myocardial changes are more pronounced in AO rats with psoriasis compared to DA rats. Cardiomyocytes have branching syncytium architecture with moderate defibrillation of cardiac fibers. Moderate extravasation of erythrocytes in the interstitium is present. The results of this study indicate that the activation of inflammatory signalling pathways in psoriasis affects the body as a whole and has repercussions on the cardiovascular system. By further analysing the results and using a different methodological approach, we will try to explain the more prominent changes in AO rats, in which the Th2 immune response dominates.

A LYSOSOMAL ION CHANNEL VARIANT THAT DISRUPTS LYSOSOMAL-TO-MITOCHONDRIAL INTERACTION IS A NOVEL GENETIC CAUSE OF SHORT QT SYNDROME

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Short QT syndrome (SQTS) is an extremely rare inherited cardiac disorder characterized by abnormally short QTc intervals and an increased risk of sudden cardiac death (SCD). Only 20% of SQTS patients have been linked to genetic mutations, typically in sarcolemmal ion channels. We investigated the role of dysfunctional endolysosomal TMEM175 channels as a potential contributor to SQTS. TMEM175 is known to regulate lysosomal pH, facilitate proper autophagy progression, and possibly influence mitochondrial activity. Parkinson's is the only disease associated with TMEM175 variants, although there is no consensus on specific pathogenic mechanisms. To investigate the molecular mechanism by which the TMEM175R377H variant results in the arrhythmogenic SQTS phenotype. Whole-exome sequencing identified a TMEM175 variant (chr 4.11:c.951899G>A; p.Arg377His) in a family with a history of short QT intervals and SCD. We generated a CRISPR-engineered mouse model carrying the same variant. A multidisciplinary approach combining patch-clamping, intracardiac stimulation, optical mapping, molecular biology techniques, and Transmission Electron Microscopy (TEM) to assess the functional and structural effects of the mutation. Mice carrying the TMEM175R377H mutation exhibited abnormal ECGs, including shortened QT interval, conduction defects, and increased arrhythmia susceptibility. TMEM175R377H cardiomyocytes showed reduced action potential duration (APD), with a slight increase in IK1 density at +20 mV. TEM revealed physical disruption of the lysosome-mitochondria axis and mitochondrial disorganization. Elevated levels of autophagy markers, including LC3, Rab5, and Lamp1, suggested mitochondrial dysfunction. Mutant mice had reduced maximal respiration, spare respiratory capacity, and ATP synthesis. This energy deficit activated the KATP+ channel, whose current (IKATP) further shortened the APD, contributing to the SQTS phenotype. Finally, we demonstrate that disruption of the TMEM175-BCL2 interaction is a key factor in mitochondrial dysfunction and the development of SQTS. In conclusion, the lysosomal protein TMEM175 plays a crucial role in maintaining lysosome-mitochondria interactions and ensuring proper cardiac function. We showed that disruption of TMEM175-BCL2 interaction leads to energy imbalances and IKATP activation, contributing to the pathology of a novel SQTS type. TMEM175R377H is the first mutation in an intracellular membrane channel associated with SQTS.

NECROPTOSIS IN AN EARLY STAGE OF POST-MYOCARDIAL INFARCTION HEART FAILURE IS LIKELY MEDIATED BY TUMOUR NECROSIS FACTOR BUT NOT INTERFERONS

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Introduction: Heart failure (HF) following myocardial infarction (MI) remains a major contributor to morbidity and mortality. Necroptosis has been recognized as one of the key mediators in the development of post-MI HF, however, its initiating mechanisms are not fully elucidated. In this context, tumour necrosis factor (TNF) is the most studied yet, but recent evidence suggests that interferons (IFNs) and other innate immune mediators might also play a role. Thus, we aimed to elucidate the potential pro-necroptotic mechanisms in an early stage of post-MI HF. As translation approach, we also measured the circulating levels of these cytokines in the patients with HF.
Methodology: Post-MI HF in rats was induced by the ligation of the left anterior descending artery (LAD) for 1 hour followed by 7-day reperfusion. Treated animals were given a pharmacological inhibitor of necroptosis, GSK´872. The heart function was assessed by echocardiography and the molecular analyses were performed by Western Blotting and ELISA.
Results: The key markers of necroptosis, receptor-interacting protein kinase 3 (RIP3) and mixed lineage kinase domain-like protein (MLKL), were significantly elevated in the post-MI failing hearts while the anti-necroptotic treatment was able to normalise them. The levels of TNF and its specific receptor, TNFR1, were upregulated alongside the downstream protein kinase, RIP1, inducing the canonical necroptotic pathway. The levels of toll-like receptors 3 and 4 (TLR3/4) and their ligand, high mobility group box protein 1 (HMGB1), were also upregulated in the diseased hearts. Contrarily, the levels of type I and II IFNs and their downstream Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling, was suppressed due to post-MI HF. In patients with HF, the serum levels of HMGB1, unlike TNF and IFN gamma, tended to increase.
Conclusion: Our findings suggest that necroptosis in the early stage of post-MI HF is likely predominantly mediated by the cytokine of TNF family, but not IFNs. Furthermore, the canonical TNF–RIP1–RIP3–MLKL pathway and TLR-mediated non-canonical signalling have been suggested to process the necroptotic stimulus. Targeting these pathways of necroptosis might be effective in the limitation of necroptosis in the diseased heart.

MAPPING THE HEART FAILURE CONTINUUM IN A NATIONWIDE POPULATION: STAGES, TRENDS, AND OPPORTUNITIES FOR PREVENTION

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Background: Heart failure (HF) remains a major contributor to morbidity and mortality worldwide. While most epidemiological studies focus on clinical HF (Stages C and D), data on preclinical stages (A and B) are limited. We aimed to develop a tool for classifying HF stages using administrative data and to evaluate the nationwide prevalence, trends, and mortality rates across these stages.
Methods: We conducted a retrospective analysis of the entire population of Czechia using data from the National Registry of Reimbursed Health Services and linked mortality records. We developed an HF stages classification based on ICD-10 codes, prescribed medication, and therapeutic procedures.
Results: Between 2015 and 2023, the absolute prevalence of all HF stages increased. Age-standardized prevalence also rose by 8.4% for Stage A and 17.8% for Stage B. Clinical HF (Stages C and D) showed a nonlinear trajectory, plateauing during and after the COVID-19 pandemic. One-year mortality increased progressively across heart failure stages, from 0.97% in Stage A to 19.45% in Stage D, with a rising proportion of cardiovascular causes and a declining proportion of oncological causes of death with advancing HF stage. Preclinical HF stages preceded the onset of clinical HF by several decades.
Conclusion: We present a novel tool for identifying HF stages in administrative data. Applied to the Czech population, this approach reveals a rising burden of both preclinical and clinical HF beyond what can be explained by population aging alone. These findings underscore the urgent need for preventive strategies, particularly those targeting Stage A, to mitigate future HF incidence.

THE COMPARISON OF THE PROPERTIES AND ROLE IN IN CARDIAC ACTION POTENTIAL OF THE ATRIAL AND VENTRICULAR TRANSIENT OUTWARD POTASSIUM CURRENT

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Background: Atrial fibrillation (AF) is a common and severe arrhythmia, which largely affects the quality of life. State of the art treatment of AF still relies heavily on pharmacological modalities. During development of new antiarrhythmic drugs for treating AF, dog AF models are used. Therefore, the aim of present study is to investigate and compare the properties the transient outward (I_{to}), in isolated ventricular and atrial myocytes obtained from normal dogs.
Methods: Transmembrane ionic currents were recorded using the whole-cell configuration of the patch-clamp technique in single atrial myocytes isolated via Langendorff perfusion methods from dog hearts. Experiments were carried out at 37 °C.
Results: A large I_{to} current was observed in dog atrial myocytes in comparison with that of observed. The current amplitude was 15.2 ± 1.1 pA/pF at 50 mV. The inactivation phase of the current was best fitted by two exponentials (fast time constant: 11.26 ± 1.16 ms, fast amplitude: 10.4 ± 0.84 pA; slow time constant: 116.13 ± 8.18 ms, slow amplitude: 3.68 ± 0.46 pA/pF; n = 10, test potential: 50 mV). The slow the time constant of I_{to} inactivation found to be much slower compared to that measured in dog ventricular myocytes (14.25 ± 1.16 ms at 50 mV, n = 12). The I_{to} current during the action potential plateau phase was measured as chromanol 293B (which inhibits Ito at high concentration) sensitive current in atrial myocytes isolated from dog hearts using an atrial action potential waveform for the command potential. It was found that I_{to} carries much larger outward current in the plateau phase of the atrial action potential than that found in ventricular cells. The effects of I_{to} blockade on the action potential repolarization were also investigated in dog atrial muscle by the standard microelectrode technique. The current was inhibited by application of 100 μM chromanol 293B. The drug increased the plateau potential but failed to lengthen the action potential in atrial preparations. Application of another I_{to} blocker (4 mM 4-aminopyridine) resulted in similar outcome. However, the amplitude and kinetics of the slow phase of I_{to} inactivation implied a substantial I_{to} current during the plateau phase of the atrial action potential, blockade of the current did not lengthen the action potential in dog atrial muscle.
Conclusions: Due to its slow inactivation kinetics the atrial I_{to} current may contribute to late repolarization in atrial myocytes to a greater extent than has been shown in ventricular myocytes. I_{to} current in our dog model, in contrast to human data, was only slightly "downregulated" in AF, but its inactivation kinetics were significantly slowed.

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THE EFFECT OF A COMPREHENSIVE CARDIAC REHABILITATION PROGRAM ON EXERCISE PARAMETERS IN PATIENTS WITH ARTERIAL HYPERTENSION

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Introduction: Arterial hypertension is a major risk factor for cardiovascular disease. Cardiac rehabilitation programs have been shown to improve exercise capacity and quality of life in patients with a cardiovascular disease.
Purpose: The aim of this study was to evaluate the effect of a comprehensive cardiac rehabilitation program on exercise parameters in patients with arterial hypertension treated in a single university level centre.
Methods: We retrospectively reviewed the records of 58 patients with arterial hypertension who underwent a comprehensive cardiac rehabilitation program for 1 year. The programme included intervention of cardiovascular disease risk factors by a cardiologist, psychologist and nutritionist. There were also 7 individual sessions with a physical therapist. There was also voluntary participation in regular group exercise. Exercise capacity was assessed by cardiopulmonary exercise testing (CPET) at baseline and after completion of the program. We also collected resting data on blood pressure, heart rate, and basic physical characteristics.
Results: The cardiac rehabilitation program resulted in a significant improvement in exercise capacity. Peak oxygen uptake (VO_{2peak}) increased from 23 (6) ml/kg/min at baseline to 27 (8) ml/kg/min after completion of the program (p <0.001). There was also a significant decrease in resting blood pressure. Systolic blood pressure decreased from 136 (10) mmHg to 132 (9) mmHg (p = 0.015), and diastolic blood pressure decreased from 87 (8) mmHg to 85 (6) mmHg (p <0.001). BMI also decreased from 30.4 (5.5) kg/m^2 to 29.1 (5.4) kg/m^2 (p = 0.025).
Conclusion: A comprehensive cardiac rehabilitation program is effective in improving exercise capacity and resting blood pressure in patients with arterial hypertension. This type of program should be considered for all patients with arterial hypertension.

PERIPARTAL BAROREFLEX SENSITIVITY IN CHRONIC HYPERTENSIVE DAMS

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Introduction: The baroreceptor reflex (BRR) is a fundamental cardiovascular mechanism operating via negative feedback, crucial for short-term blood pressure (BP) regulation and contributing to long-term homeostasis. BRR dysfunction is observed in both experimental and essential hypertension, while reduced baroreflex sensitivity (BRS) is a known predictor of morbidity and mortality in cardiovascular diseases. As part of the cardiovascular adaptations to normotensive pregnancy, BRS decline may elevate the risk of peripartum haemorrhage. However, data on BRS in chronically hypertensive parturients remain limited and inconclusive.
Method: Female Wistar rats (WR) and Spontaneously hypertensive rats (SHR), a genetic model of hypertension, were monitored using implanted radiotelemetry probes at baseline (diestrus, DE), late pregnancy (LP), and postpartum days 1 (P1) and 5 (P5). Systolic (SBP) and Diastolic (DBP) BP and heart rate (HR; derived from inter-beat intervals) were assessed from arterial waveforms. BRS was assessed using the sequence method in BP Complete software. A two-way mixed ANOVA was conducted to assess the effect of strain and time, while a one-way ANOVA was used for between-group comparisons.
Results: Compared to WR, SHR rats displayed significantly elevated SBP (b = 49.32, p <0.001) and DBP (b = 28.65, p = 0.007) along with lower HR (b = –21.59, p = 0.03), persisting across time points. Intragroup testing revealed a significant drop in LP SBP (p = 0.01; p = 0.001) in both groups with increased HR (p = 0.03) only observed in WR. BRS increased over time (b = 0.08, p = 0.03), with no significant differences between strains. SHR showed a transient BRS increase at LP (p = 0.007) returning to baseline by P5. WR exhibited a declining BRS trend during LP and a near-significant rebound at P5 (adjusted p = 0.06).
Conclusion: WR dams experienced pregnancy-induced BP reductions with compensatory HR elevations, normalizing postpartum. The BP drop was more pronounced in SHR, nearing normotensive levels at term, accompanied by a BRS increase. The transient increase in BRS implicates a temporary enhancement of autonomic cardiovascular control despite chronic hypertension. This adaptive parasympathetic response may serve a protective role even in pregnant individuals at cardiovascular risk. WR rats, in contrast, showed postpartum BRS augmentation, suggesting potential long-term cardiovascular benefits of pregnancy in normotensive females.

OLIVE LEAF EXTRACT AND LOSARTAN BLUNTED FIBROSIS BY DOWNREGULATING BETA-CATENIN/FIBRONECTIN PATHWAY IN HYPERTENSIVE RATS WITH CHRONIC KIDNEY DISEASE: IS WNT4 RESPONSIBLE?

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Introduction: Wnt proteins are highly conserved signaling molecules that play an essential role in renal development. Through modulating cell adhesion and migration, Wnt4 regulates the mesenchymal-to-epithelial transition, tubulogenesis, and promotes nephron elongation and differentiation. However, Wnt/β-catenin signaling is reactivated in tubulointerstitial fibrosis, podocyte injury and dysfunction, contributing to the pathogenesis of proteinuria and glomerulosclerosis. Beta-catenin stimulates the transcription of genes that are implicated in matrix production and fibrosis, such as fibronectin. Here, we investigated if adding of olive leaf extract (O, natural antioxidant rich in phenolic compounds) to losartan treatment (L, angiotensin II type-1 receptor blocker) could be more effective in preventing renal fibrosis and slowing down the progression of adriamycin (ADR)-induced focal segmented glomerulosclerosis (type of chronic kidney disease) in spontaneously hypertensive rats (SHR).
Methodology: SHRs were divided into four groups. Control received vehicle. Three groups received ADR (2 mg/kg, i.v.) twice in a 3-week-interval, and two groups received L, and L+O (10, and 10 + 80 mg/kg/day, respectively) by gavage for 6-week-period. Renal Wnt4, β-catenin and fibronectin protein expressions were analyzed by Western blot.
Results: In the kidney of model group, we found significantly reduced Wnt4 followed by significantly increased β-catenin and fibronectin protein expressions compared to control group. Losartan normalised Wnt4 expression with no other effects. However, losartan in combination with olive leaf extract normalised Wnt4 protein expression and significantly decreased β-catenin compared to model group which was followed by significant fibronectin reduction to the level not significantly different from control group.
Conclusion: Our results showed that losartan treatment supplemented with olive leaf extract downregulated β-catenin/fibronectin signaling pathway and abolished fibrosis in the kidney of SHR with ADR-induced nephropathy, at least partly, by normalizing Wnt4 protein expression.

ADIPOSE TISSUE LIPID REMODELING IN HEART FAILURE

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Background: Heart failure (HF) with reduced ejection fraction involves adipose tissue metabolic adaptations poorly understood in epicardial (EAT) and subcutaneous (SAT) depots. This study explores lipid metabolism, fatty acid oxidation (FAO), and ketogenesis across HF stages.
Methods: Clinical/metabolomic profiling of controls (n = 34), mild HF (n = 45), and severe HF (n = 121) patients. Liquid chromatography-mass spectrometry analyzed >800 metabolites in EAT/SAT, supplemented by pathway/gene expression analyses.
Results: HF progression correlated with cardiac decline, systemic congestion, and a catabolic shift. SAT showed gradual lipolysis activation, while EAT exhibited impaired triacylglycerol replenishment and delayed β-oxidation. Both depots increased acylcarnitine utilization, but EAT uniquely accumulated dicarboxylic acylcarnitines and downregulated peroxisomal FAO genes. Severe HF upregulated ketogenesis, particularly in EAT (enhanced in cachexia), with *ex vivo* confirmation of β-hydroxybutyrate production. Both depots redirected fatty acids/branched-chain amino acids (BCAAs) toward ketones, with EAT compensating via BCAA degradation.
Conclusions: HF drives depot-specific adipose remodeling. EAT in advanced HF displays impaired FAO and heightened ketogenesis, exacerbated in cachexia, suggesting maladaptive metabolic reprogramming contributes to systemic energy dysregulation and cardiac cachexia.

CARDIOPROTECTION BY REMOTE ISCHEMIC CONDITIONING: RED BLOOD CELL-MEDIATED REDUCTION OF MYOCARDIAL INFARCT SIZE AND CORONARY NO-REFLOW

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Introduction: Cardioprotection research has primarily focused on infarct size reduction. Microvascular damage after acute myocardial infarction, however, also determines patient prognosis. Remote ischemic conditioning (RIC), i.e. brief cycles of ischemia and reperfusion (I/R) in tissues remote from the heart, triggers the release of humoral factors that confer cardioprotection. The protective effect of RIC can be transferred from conditioned donors to naïve *ex vivo* hearts subjected to I/R by infusing plasma or washed platelets. However, not only platelets, but also red blood cells (RBC)s and the RBC-derived nitric oxide (NO) play a role in cardioprotection. In isolated, perfused hearts with I/R, RBCs reduced myocardial infarct size. We now aim to identify whether RBCs carry a cardioprotective signal activated by RIC and, if so, whether this signal also protects the coronary microcirculation.
Methods: Healthy volunteers underwent RIC (3 × 5/5 min upper-arm I/R at 200 mmHg). Venous blood samples were collected before and 60 minutes after RIC. RBCs were separated by centrifugation, and incubated with saline, saline plus arginase (4 U/mL), or saline plus oxyhemoglobin (0.1 mol/L), respectively. RBCs (5×10^{12}/mL) were infused into isolated, perfused rat hearts for 8 minutes, followed by a 2-minute washout period. Saline infusion served as control. After 30 minutes of left anterior descending coronary artery occlusion and 120 minutes of reperfusion, the area at risk was demarked using patent blue dye, microvascular injury (no-reflow) was visualized with thioflavin-S, and infarct size was determined using triphenyltetrazolium chloride. No-reflow and infarct size were expressed as a percent of the area at risk.
Results: In the absence of RBCs, infusion with saline resulted in an infarct size of 45 ± 9% and a no-reflow of 9 ± 4%. Infusion of RBCs taken before RIC reduced infarct size to 34 ± 11%, arginase and oxyhemoglobin abrogated this infarct size reduction (44 ± 8% and 44 ± 3%), respectively. No-reflow was not modified by infusion of RBCs before RIC (12 ± 6%). Infusion of RBCs after RIC further reduced infarct size to 20 ± 9%, and decreased no-reflow to 5 ± 2%. Arginase and oxyhemoglobin did not modify the RIC mediated effect on infarct size and no-reflow.
Conclusion: RIC enhanced NO-independently RBC's cardioprotective potential and extended it to the coronary microcirculation. Thus, RBCs may serve as a unique novel target for delivering protection to the microcirculation.

MATRIX METALLOPROTEINASES AS AN EARLY-STAGE MARKERS OF CARDIAC DAMAGE IN CANINE HEARTWORM DISEASE

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Introduction: Dirofilaria immitis, a zoonotic nematode, causes canine heartworm disease (CHW). In dogs, it is characterized by heart and lung tissue damage, specifically in the associated blood vessels. The pre-adult parasite stages can induce cystic formations in the pulmonary arteries of human patients, often misinterpreted as tumors. The disease in humans is considered to be of low prevalence and mostly asymptomatic. Matrix metalloproteinases (MMPs) coordinate tissue repair and have a key role in numerous vascular disorders. The aim of this study was to evaluate levels of matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) in dogs with an early stage of CHW.
Methodology: First group included seven dogs with moderate (class 2/4) CHW. The healthy group included nine CHW negative dogs. For all patients, complete blood count and lactate dehydrogenase (LDH) were determined. To evaluate microfilaria/dirofilaria status of the included dogs, modified Knott's and commercial ELISA tests were performed. Zymography electrophoresis was used for MMP-2 and MMP-9 detection. The signals were analyzed in TotalLab TL120® commercial software. Statistical analysis was performed using Mann–Whitney U test (MedCalc software version 14.8.1.).
Results: Both groups had leukocytes within the reference range. There was no difference in LDH levels between groups (U = 22, z = 0.59, p = 0.556). The results showed significantly higher zymogen (pro)MMP-9 levels in the CHW group (U = 4, z = 2.85, p = 0.004), compared to the healthy control. The total, active MMP-9 forms (U = 28, z = 0.37, p = 0.711), as well as MMP-2 levels (U = 26, z = 0.58, p = 0.560) had similar values in both groups.
Conclusion: It could be speculated that, although there was no leukocytosis found in dogs with CHW, leukocytes do release substantially higher levels of zymogen (pro) MMP-9. The lack of difference in LDH and MMP-2 levels indicates a preserved cardiac tissue and endothelium in dogs with class 2 CHW. Nevertheless, higher release of zymogen (pro)MMP-9 from CHW dogs' leukocytes suggests their compensatory response to D. immitis infection and candidates MMPs as a potential early marker for cardiac damage monitoring in the infected dogs.

ANTI-INFLAMMATORY EFFECTS OF POLYPHENOL-ENRICHED HYDROGELS IN DIABETIC WOUND HEALING

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Background and aim: Chronic diabetic wounds are marked by sustained inflammation and oxidative stress, leading to delayed healing and poor outcomes. Conventional treatments often fail to address the underlying inflammatory dysfunction. This study investigates a novel biopolymer-based hydrogel formulation enriched with plant-derived polyphenols, which serves as a bioactive dressing capable of modulating inflammation in diabetic wound environments.
Materials and methods: To assess anti-inflammatory potential, a GSE-loaded alginate-gelatin hydrogel (HG+GSE) was topically applied to excision wounds in streptozotocin-induced diabetic rats. Inflammatory responses were monitored over 15 days by measuring serum concentrations of TNF-α and IL-6 using ELISA. These were compared against control groups receiving hydrogel base, 1% silver sulfadiazine, or no treatment.
Results: The HG+GSE formulation significantly reduced systemic levels of IL-6 and TNF-α at all measured time points compared to controls. This modulation of inflammatory cytokines was accompanied by enhanced histological tissue organisation and re-epithelialization. The treatment also exhibited a favourable antioxidant profile and was well-tolerated, with no dermal irritation.
Conclusion: The results support the use of polyphenol-enriched biopolymer hydrogels as a safe and effective strategy for controlling inflammation in diabetic wounds. By targeting key pro-inflammatory pathways, this novel dressing may accelerate healing and reduce the risk of chronic wound complications.

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THE 'TWO-HIT' MOUSE MODEL OF LV DIASTOLIC DYSFUNCTION: RELEVANCE TO HFPEF

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Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome with rapidly increasing inci-

dence and prevalence but limited therapeutic options. A detailed understanding of the complex pathophysiological mechanisms underlying diverse clinical phenotypes is dependent on the availability of appropriate and sufficiently robust experimental animal models. One of the most studied mouse models of cardiometabolic HFpEF is the 'two-hit' model combining obesity and hypertension as major comorbidities induced in C57BL/6N mice fed a high-fat diet and water supplemented with NO-synthase inhibitor. It has been shown to recapitulate some key features of the clinical syndrome already after 5 weeks of treatment. We attempted to introduce this model with the initial aim of assessing the potential role of HIF-1α in the pathogenesis of HFpEF. However, we observed only glucose intolerance (potentiated by HIF-1α deficiency), mild cardiac hypertrophy and reduced exercise capacity, but neither left ventricle diastolic dysfunction (LVDD) nor pulmonary edema in FVB and C57BL/6N males after 8 weeks of treatment. Extending treatment to 16 weeks resulted in a significant increase in diastolic parameters (IVRT, E/A, E/E´), but not in myocardial fibrosis and pulmonary edema in C57BL/6N females. The degree of exercise intolerance correlated closely with obesity but not with LVDD. Thus, in our study, the 'two-hit' model led to diastolic dysfunction without a clear manifestation of HFpEF. The different mouse strains used, sex-related differences as well as other factors may contribute to the inconsistent results reported in the literature.

UNDERMINING THE MOLECULAR ROOTS OF ATHEROSCLEROSIS: IN SILICO SCREENING OF COMMERCIALLY AVAILABLE DRUGS AND THEIR NATURAL ANALOGS TARGETING DKK2 PROTEIN – MOLECULAR DOCKING AND ADME-BASED EVALUATION

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Introduction: DKK2, a member of the Dickkopf protein family and a known antagonist/agonist of the Wnt/β-catenin signaling pathway, has emerged as one of the significant modulator in the pathogenesis of atherosclerosis. Dysregulated DKK2 expression has been identified in endothelial cells, macrophages, and vascular smooth muscle cells (VSMCs) within atherosclerotic plaques. In endothelial cells, DKK2 impairs angiogenic and regenerative processes by suppressing β-catenin-mediated gene transcription, leading to increased vascular permeability and reduced repair capacity. In macrophages, DKK2 enhances lipid uptake and inflammatory signaling, contributing to foam cell formation. Moreover, DKK2 stimulates VSMC proliferation and migration, facilitating plaque progression and destabilization. Given its involvement in multiple pathogenic processes, DKK2 represents a promising but underinvestigated molecular target for anti-atherosclerotic drug development. This study aimed to explore interactions between DKK2 and a wide range of pharmacologically active compounds through molecular docking and pharmacokinetic profiling, followed by the *in silico* generation of natural product-based analogues with enhanced binding potential.
Methodology: A virtual screening of 4500 approved small molecules was conducted using
AutoDock Vina, employing the crystallographic structure of DKK2 obtained from UniProt and the PDB. Ligands were ranked by Gibbs free energy (ΔG <–7.0 kcal/mol) and RMSD < 3.0 Å. The top 10 most potent drugs were used further to generate 100 analogues each using DataWarrior, yielding a total of 1000 new compounds from the specter of natural compounds. These were redocked to DKK2. Pharmacokinetic profiles were assessed via SwissADME, evaluating Lipinski's rule, rotatable bonds, logP, TPSA, GI absorption, and BBB penetration.
Results: Among the initial compounds, 41 exhibited high binding affinity for DKK2, with deptropine, lasofoxifene, antrafenine, azithromycin, and efonidipine showing the strongest interactions (ΔG ≤ –10.3 kcal/mol). Among the generated analogues, 44 difenadione-based structures showed improved binding than initial compound. Seven natural analogues met all ADME criteria and were prioritized for further study.
Conclusion: This dual-phase in silico investigation identified multiple DKK2-binding ligands with favorable pharmacokinetic characteristics, supporting their further evaluation as scaffolds for novel anti-atherosclerotic therapies.

NEAR DEATH EXPERIENCES (NDE) IN PATIENTS WITH REFRACTORY CARDIAC ARREST TREATED WITH CONVENTIONAL AND EXTRACORPOREAL CARDIOPULMONARY RESUSCITATION IN LIGHT OF FURTHER RESEARCH

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Objective: Near death experiences have not yet been studied in patients with refractory cardiac arrest treated with

extracorporeal cardiopulmonary resuscitation (ECPR). We determined the prevalence of NDEs in patients from the randomized Prague OHCA study with refractory cardiac arrest and analyzed differences between patients resuscitated with standard cardiopulmonary resuscitation (CPR) and those resuscitated using the ECPR method.
Methods: NDEs were analyzed using a standardized and internationally validated questionnaire among long-term survivors of the randomized Prague OHCA study. The prevalence of NDEs was compared between patients treated with standard CPR and those resuscitated with the ECPR method.
Results: The questionnaire was completed by 44 out of 60 patients who were followed up long-term after the Prague OHCA study (median age: 57.5 years, 83% male, average CPR duration: 37.5 minutes), including 12 patients treated with ECPR (average cardiac arrest duration: 54 minutes). At least one symptom of NDE was experienced by 15/44 patients (34.1%), including 10/32 (31.3%) in the standard CPR group and 5/12 (41.6%) in the ECPR group (p = 0.52). Criteria for a complete NDE (score ≥28 on the NDE questionnaire) were met by 9/44 patients (20.5%), including 6/32 (18.8%) in the standard CPR group and 3/12 (25%) in the ECPR group (p = 0.65).
Conclusion: The prevalence of NDEs in our cohort of patients with refractory cardiac arrest corresponds to the prevalence reported in previous studies that analyzed patients treated with standard CPR and shorter durations of cardiac arrest. The numerically higher occurrence of NDEs in patients treated with the ECPR strategy is hypothesis-generating but did not reach statistical significance. Further conclusions require analysis in larger patient cohorts. We proposed theories regarding the occurence or non-occurence of NDEs in light of our further research.

PREVENTIVE CARDIOLOGY: A NEW ROLE OF HIGH SENSITIVE TROPONIN I

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Aim: To introduce and to estimate the effectiveness of a high-sensitive troponin-I (hsTnI) guided screening program for cardiovascular disease (CVD) and evaluate potential clinical and health consequences.
Background: Existing tests for assessing the risk of cardiovascular diseases such as Framingham Heart Study in U.S. (FHS) or HEARTSCORE I and HEARTSCORE II, and HEARTSCORE II-OP in Europe have been in use for a very long time, and conclusion is that elevated cholesterol and blood pressure are the main risk factors for CVD. On the other hand, HEARTSCORE's calculate the 10-year risk of fatal and non-fatal cardiovascular events, but these scores are applicable for healthy individuals only. So, HEARTSCORE I and HEARTSCORE II-OP should not be used for people who have proven atherosclerotic cardiovascular disease, high risk conditions, diabetes, familiar hypercholesterolemia, genetic or rare diseases of blood lipids or arterial hypertension, chronic kidney disease or in pregnant women.
Results: A study of asymptomatic women and men between 40–70 years old, with no specific symptoms and no confirmed or known coronary artery disease who voluntarily participated in a risk assessment and screening program for CVD in Zagreb, was performed. Participants were stratified into three risk categories according to their CVD risk factor hsTnI level. Subjects in the moderate and high-risk class were referred to further non-invasive cardiovascular diagnostic test and coronary angiography or PCI (Percutaneous Coronary Intervention), if required. In the model, this program reduced the incidence of acute CVD events by 180 per 10,000 subjects, equal to a number-needed-to-screen of 56. CVD-related mortality decreased by 40%.
Conclusion: Screening asymptomatic female and men subjects with hsTnI and guiding those at higher risk to further cardiac testing, identified subjects with coronary artery disease and referred those at high risk to further diagnostic tests. In a cost-effectiveness analysis, this strategy might reduce the CVD related burden and mortality and would likely be cost-effective.

THE COMPARISON OF ECHOCARDIOGRAPHIC PROFILES IN PATIENTS WITH AL AND WILD-TYPE ATTR CARDIAC AMYLOIDOSIS AT THE TIME OF DIAGNOSIS

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Background and aim of the study: The two most common types of cardiac amyloidosis (CA) are light-chain (AL) and transthyretin (ATTR) amyloidosis. Echocardiography is a fundamental diagnostic tool in the initial evaluation of CA. Little is known about differences in echocardiographic characteristics between both diseases possibly related to their specific aetiology. Therefore, the aim of our study was to compare echocardiographic findings in newly diagnosed patients with AL and wild-type ATTR CA, respectively.
Methods: We retrospectively evaluated initial echocardiographic parameters in a cohort of newly diagnosed patients with AL (n = 50, years 2010–2024) and wild-type ATTR (n = 50, years 2023–2024). In all cases, the diagnosis of CA was made according to current recommendations. Data are expressed as a mean ± SD or as a percentage of subjects.

Results: There was no statistically significant difference in interventricular septal wall thickness (16 ± 3 mm vs. 17 ± 3 mm), left ventricular end-diastolic diameter (46 ± 7 mm vs. 47 ± 6 mm), left ventricular mass (152 ± 51 g/m^2 vs. 157 ± 48 g/m^2), left ventricular ejection fraction (56 ± 12% vs. 56 ± 9%), global longitudinal strain (10 ± 3 mm vs. 11 ± 3mm) between patients with AL and ATTR, respectively. In AL subjects, E/e´ (23 ± 12 vs. 19 ± 8), pulmonary artery systolic pressure (39 ± 11 mmHg vs. 35 ± 11 mmHg) and estimated right atrial pressure (9 ± 6 mmHg vs. 6 ± 5 mmHg) were significantly higher compared to ATTR patients (p <0.05 for all). The presence of pericardial effusion was also more frequent in AL subjects (76% vs. 36%, p <0.01).
Conclusions: Although there are no differences in left ventricular morphological and systolic characteristics, the patients with AL CA express more advanced impairment of filling of both ventricles at the time of diagnosis, associated with higher pulmonary artery pressures and more frequent presence of pericardial effusion. These echocardiographic findings reflect more advanced stage of heart failure in patients with AL CA at the time of diagnosis.

CHRONIC LEMON BALM EXTRACT ADMINISTRATION MODULATES THE PROGRESSION OF DILATED CARDIOMYOPATHY IN EXPERIMENTAL AUTOIMMUNE MYOCARDITIS

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Introduction: *Melissa officinalis* (lemon balm) is long-known medicinal plant, rich in phenolic acids and flavonoids with notable therapeutic potential. The goal of this study was to evaluate the possible role of ethanolic lemon balm extract (LBE) in preventing cardiac remodelling and subsequent dilative cardiomyopathy (DCM) in chronic experimental autoimmune myocarditis (EAM).
Methodology: A total of 50 male Dark Agouti rats (8 weeks old, 150 ± 20 g) were randomly divided into five groups: healthy control (CTRL), EAM, and three EAM groups treated with LBE at doses of 50, 100, or 200 mg/kg orally (LBE-S, LBE-M, LBE-H), once daily for 42 days. On day 0 of the protocol, EAM was induced by porcine cardiac myosine intraplantary injection (immunization dose 0.25 mg). After finishing the protocole, echocardiographic measurements were performed, prior to sacrification of animals. Serum samples were used for determination of TGFβ by ELISA method, while heart tissue was analyzed by hematoxylin and eosin (H/E) staining for inflammatory infiltration and Sirius Red staining for fibrosis and collagen content.
Results: Echocardiographic analysis confirmed the development of dilated cardiomyopathy (DCM) in EAM rats, evidenced by significantly reduced ejection fraction (EF), fractional shortening (FS), and left ventricular posterior wall (LVPW) thickness. LBE treatment, particularly at the highest dose (LBE-H), significantly improved these parameters. Six-week LBE administration significantly reduced serum TGF-β levels (p <0.05) compared to untreated EAM rats, and preserved cardiac structure. Sirius Red staining revealed significantly lower collagen deposition in LBE-treated groups (p <0.05), while H/E staining showed reduced inflammatory infiltrates compared to untreated EAM hearts.
Conclusion: Lemon balm extract (LBE) shows promising cardioprotective potential by preserving cardiac structure, reducing fibrosis, and improving hemodynamic parameters in DCM pathology following chronic phase of EAM. LBE may serve as a valuable phytotherapeutic agent in the management of autoimmune myocarditis

RECENT ADVANCES IN NON--PHARMACOLOGICAL STRATEGIES AIMED TO TRIGGER INNATE CARDIOPROTECTION IN HEALTHY AND DISEASED MYOCARDIUM

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The incidence of cardiovascular diseases, especially ischemic heart disease (IHD) and acute myocardial infarction (AMI), leading further to heart failure, will not reduce over the coming decades, and there is a need for novel approaches that could address the regeneration of myocardium. Ischemic preconditioning (IPC) is the most robust adaptive phenomenon protecting the heart of all animal species against ischemia/reperfusion (I/R) injury. However, its application in humans is limited by technical requirements (chest opening), short-term protection duration, unpredictable AMI occurrence, and it can be used only in elective interventions. Protection against I/R can be rendered by other forms of "conditioning", such as "remote" PC (RPC), in which ischemia of any organ confers protection to the other, distant organs/tissues, or exercise PC (EPC) that do not require an invasive intervention.
We explored whether preventive interventions applied *in vivo* increase cardiac resistance to I/R prior to AMI *ex vivo* using non-invasive approaches in the adult male Wistar rats: voluntary exercise (2 weeks free running in the

wheel-equipped cages) and RPC, second phase (24 h after application) induced by 3 cycles of 5 min inflation (200 mmHg)/5 min deflation of pressure cuff on the hind limb. The efficacy of EPC and RPC was tested in the isolated Langendorff-perfused normo- and hyperglycemic hearts exposed to 30 min global ischemia/2 hrs reperfusion, focused on the post-I/R recovery of function (LVDP), arrhythmogenesis and extent of lethal injury (infarct size, IS, TTC staining). In parallel groups, heart tissue samples were obtained for the investigation (WB) of the levels and activity of several proteins involved in "pro-survival" RISK pathway and pro- and anti-apoptotic effects.
Both EPC and RPC significantly reduced contractile dysfunction, IS, and incidence and severity of reperfusion arrhythmias. These effects were observed in both normo- and hyperglycemic hearts, but were less expressed in the hyperglycemic hearts. Protective effects were associated with a significant up-regulation of selected RISK proteins, such as PKB (reduced by hyperglycemia), PKCα, eNOS, higher levels of SOD, HSP, and reduction of pro-apoptotic cascades (BAX/Bcl-2, Caspase-3).
The beneficial effects of non-invasive forms of PC suggest their potential in the management of IHD in clinical conditions. Potential mechanisms may involve activation of proteins of RISK cascades.

EFFECT OF RIGHT VENTRICULAR PACING LEAD SITE ON MORTALITY – RESULTS OF PILOT STUDY

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Introduction: Cardiac pacing from the right ventricular apex (RVA) is associated with dyssynchronous electrical activation and contraction. Long-term apical pacing (RVAP) could lead to an increased risk of death, heart failure, and atrial fibrillation. Right septal pacing (RVSP) offers more physiological electrical activation. However, its effect on long-term clinical outcomes is still unknown. Previous clinical studies examining mortality have provided inconsistent results. The aim of this prospective single-center study is to compare all-cause mortality between RVSP and RVAP. Here we present pilot data from 4-year follow-up.
Methods and results: In this pilot study we evaluated 539 patients (age 76,8 ± 9,9 years, 60.7% male), who underwent pacemaker implantation in years 2019 and 2020 in our cardio-centre. The patient received pacing at RVA (338), low-RVS (165), or mid-RVS (35) according to implanting physician decision. At 4 years, 153 (28.4%) patients died, 106 (31.4%) in the RVA group, 36 (21.8%) in the low-RVS group (p = 0.46), 11 (31.4%) in the mid-RVS group (p = 0.23). There was no significant difference in mortality between RVA and low-RVS or mid-RVS position. Age, male gender and higher percentage of pacing were significant predictors of mortality. DDD stimulation was associated with better survival.
Conclusion: In this pilot study there was no significant difference in mortality between RVA, low-RVS, or mid-RVS right ventricular lead position. These results suggest that simple septal (i.e. non-conduction system) pacing does not influence prognosis. Further analyses are necessary.

ISOPRENALINE-INDUCED CHRONIC HEART FAILURE IS ALLEVIATED BY PYRIDOSTIGMINE TREATMENT IN RATS

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Background/aim: Autonomic imbalance is a key pathological disturbance in chronic heart failure (CHF). In-

creased oxidative stress and inflammation are considered primary factors contributing to disease progression. Growing evidence indicates that cholinergic stimulation may serve as a potential therapeutic strategy for CHF, as it helps restore autonomic balance and modulates the inflammatory response through the cholinergic anti-inflammatory pathway. While previous studies have shed some light on the mechanisms behind these effects, there remains a gap in understanding regarding the various methods of cholinergic stimulation and their specific modes of action.

Methodology: In this study, an isoprenaline model was employed (1 mg/kg/day subcutaneously for 7 days to induce CHF, followed by 4 weeks of disease development). Subsequently, rats were administered pyridostigmine (22 mg/kg/day in drinking water for 14 days) or received no treatment. Electrocardiography and echocardiography were performed before intervention, on day 7 of isoprenaline/saline administration to confirm CHF and on the end of the treatment. Samples were collected for the assessment of antioxidative status, NT-proBNP, MMP-2 and MMP-9 levels, as well as for histopathological analysis.

Results: Pyridostigmine effectively halted CHF progression, evidenced by decreased chamber wall thinning (↑ PWDd, ↑ PWDs) and reduced left ventricle dilatation (↓ LVIDd, ↓ LVIDs), along with improvements in cardiac contractile function (↑ EF). Furthermore, pyridostigmine enhanced antioxidative status (↓ TBARS, ↓ NO_2–; ↑ CAT, ↑ GSH) and significantly limited cardiac fibrosis, as confirmed through histopathological analysis and reductions in biochemical markers (↓ MMP2, ↓ MMP9).

Conclusion: Pyridostigmine effectively halted CHF progression. Nonetheless, further research is necessary to fully elucidate the precise cellular mechanisms by which pyridostigmine attenuates CHF.

REACHING TARGET FUNDAMENTAL DRUG DOSES IN PATIENTS WITH CHRONIC HEART FAILURE: RESULTS OF THE MULTICENTRIC OBSERVATIONAL PROSPECTIVE COHORT STUDY OF THE HEART FAILURE OUTPATIENTS MANAGEMENT IN THE CZECH REPUBLIC (MOST HF)

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Background: Reaching maximal tolerated fundamental drug doses in patients with heart failure and reduced ejection fraction is a key principal of treatment optimisation. Multicentric prospective cohort study focused on the management of care in outpatients with chronic heart failure in Czechia.

Study objective: To evaluate the level of medical therapy and drugs dosed in patients with HFrEF and patients with heart failure with improved ejection fraction (HFimpEF) in the MOST HF registry.

Patients and methods: Medical therapy was analysed from the database of participants of the multicentric, prospective, non-interventional study with data collection in the conditions of ordinary practice in outpatients cardiology clinics.

Results: Between 1.6.2022 and 30.9.2024 total number of 1050 patients from 16 centers were included in the study, 73.4% males and 26.6% females, mean age 64.5 years (median 66). Of 38.1% had dilated cardiomyopathy and of 37.4% had CAD, with history of MI 29.8%, T2DM 35.1%, COPD 17% and CKD 31.4%. Of 41.4% patients had history of any type of atrial fibrillation, of whom 40.9% underwent afib ablation. HFrEF was a phenotype in 68.3% subjects (n = 716), and HFimpEF in 13.8% (n = 145). At time of study entry, of 17.9% patients were in NYHA I, NYHA II 58.3%, NYHA III 22.7% and NYHA IV 1% subjects. Mean LV EF was 36.8% (median 35%), sinus rhythms was present on ECG in 58.9% of patients, paced rhythm in 36.7% and afib in 14.2% of patients. Combination of four fundamental drug groups was used by 57.1% of HFrEF patients, and by 48.8% of HFimpEF subjects. BBs were used by 94.5% patients (in HFrEF 95% and 95.2% in HFimpEF). Target BB dose was reached in 17.8% of subjects. ARNI were used by 57.4% of subjects (by 69.7% of HFrEF and by 47.6% by HFimpEF), target ARNI does was identified in 11.6% patients. MRAs were used in 81.1% patients, target MRA dose in 24.3 %. Inhibitors of SGLT2 were used in 66.8% patients, in HFrEF 74,7%, in HFimpEF 53.1%.

Conclusion: High proportion of HFrEF and HFimpEF patients is receiving optimal medical therapy. There are reserves in achieving target recommended doses of fundamental drugs in HFrEF and HFimpEF.

THE ROLE OF MITOCHONDRIAL DAMAGE-ASSOCIATED MOLECULAR PATTERNS IN NECROSIS-LIKE CELL DEATH FOLLOWING MYOCARDIAL INFARCTION-INDUCED HEART FAILURE

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Introduction: Mitochondrial DNA (mtDNA), a circular genome in the mitochondrial matrix, encodes oxidative phosphorylation components and is highly susceptible to oxidative damage. During stress-induced cell death, particularly necroptosis mediated by receptor-interacting protein kinase 3 (RIP3) and mixed lineage kinase domain-like pseudokinase (MLKL), mtDNA may be released into the cytosol or extracellular space, where it acts as a damage-associated molecular pattern (DAMP). It activates innate immune pathways and the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, triggering pyroptosis and secretion of interleukin-1β (IL-1β) and interleukin-18 (IL-18). MtDNA can easily undergo oxidation to produce oxidized mtDNA (ox-mtDNA), which further promotes inflammation and inflammasome activation. Although these mechanisms are linked to cardiac injury, the roles of mtDNA and ox-mtDNA in post-myocardial infarction heart failure (post-MI HF) remain unclear, partly due to limited and non-standardized detection methods.
Methodology: Post-MI HF was induced by the left anterior descending artery ligation for 1 hour, followed by 1 week of reperfusion in adult male Wistar rats. Following heart dissection, the entire free left ventricular (LV) wall was collected from sham-operated animals, whereas in the heart failure (HF) group, the LV was separated into the infarcted region (HFi) and the adjacent non-infarcted tissue (HFni). Protein expression was analysed by immunoblotting.
Results: Necroptotic markers pThr231/Ser232-RIP3, total RIP3, pSer345-MLKL, and total MLKL were all significantly upregulated in HFi. Pyroptotic protein levels of NLRP3, procaspase-1, and caspase-1 showed nonsignificant elevations in the same group. Both gasdermin D and its N-terminal fragment, as a terminal pyroptotic protein, displayed elevated expression in HFi. ProIL-1β levels remained constant, in contrast to mature IL-1β, which was significantly increased in the infarcted region.
Conclusion: Cell loss in the post-MI HF can occur due to necroptosis and pyroptosis. Although mtDNA appears to be a key mediator of cell death signalling, the role of mtDNA and ox-mtDNA in post-MI HF and its connection to necroptosis and pyroptosis will be the focus of our upcoming investigation.

ROLE OF BIOMARKERS OF CARDIAC AND VASCULAR REMODELING IN THE ASSESSMENT OF VOLEMIA STATUS IN PATIENTS WITH CHRONIC HEART FAILURE

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Background: Volemia status assessment is important in the management of the patients with heart failure.
Study objective: To evaluate correlation of biomarkers of cardiac and vascular remodeling concentration with parameters of volemia status as assessed by echocardiography
Patients and methods: Patients from a tertiary care heart failure clinic were examined by echocardiography and biomarkers of cardiac and vascular remodeling NT-proBNP, copeptin, cystatin-C, apelin, ADMA (asymetric dimethyl-arginin), MR-proADM (midregional pro-adrenomedulin) and sMCAM/sCD146 (soluble melanoma cell adhesion molecule) were measured. The hemodynamic parameters and volemia status were assessed by echocardiography with: EF LV, E/E´ ratio, LAVi, TAPSE, pulmonary ACT, estimated PAP) and inferior vena cava collapsibility index VCIi. Patients were followed for 18 months to assess the role of volemia status in patients' outcome.
Results: Total number of 79 patients with mean age 66 years and NYHA II–IV were included in the study. The mean LVEF was 28%, E/E´12.9, LAVi 51.6 mm/m^2, TAPSE 18.4 mm, ACT 99.8 ms, ePAP 42.3 mmHg a VCIi 32.7%. The levels of NT-proBNP and copeptin correlated significantly with parameters E/E´ ratio, LAVi, TAPSE, ePAP, and inversely with LVEF, pACT and VCIi (p <0.001 to <0.01). The concentration of cystatin C correlated with LAVi and TAPSE and inversely with ACT and VCIi (p <0.01), ADMA level correlated with VCCi (p = 0.041). Parameters LAVi, E/E´ and TAPSE were significantly higher, and LVEF and VCCi were significantly lower in patients who died or were admitted to hospital at 18 months follow-up compared to patients without event (p <0.001 to <0.01).
Conclusion: Parameters of volemia status predicted patients' outcome and biomarkers NT-proBNP, copeptin, and cystatin C were potentional indicators of volemia status in heart failure.

SEX-SPECIFIC DIFFERENCES IN PRO-INFLAMMATORY AND OXIDATIVE STRESS RESPONSES IN PRESSURE OVERLOAD-INDUCED CARDIAC DAMAGE

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Introduction: Pressure overload represents a key trigger of pathological cardiac remodelling, marked by hypertrophy, fibrosis, and a gradual loss of cardiac function. Nevertheless, the exact molecular pathways that sustain tissue damage and functional deterioration remain incompletely understood.
Methodology: To induce cardiomegaly, we used a neonatal rat model of abdominal aortic constriction (AAC), initiated on postnatal day 2 in both male and female rats. At day 90, cardiac function was evaluated using echocardiography, and molecular analysis of the left ventricle was conducted by immunoblotting to assess inflammation and oxidative stress.
Results: Both sexes showed a decrease in fractional shortening and ejection fraction post-AAC. Regarding a pro-inflammatory environment, we found increased protein expression of tumour necrosis factor (TNF), interleukin 6 (IL-6), and cyclooxygenase 2 (COX-2) only in females, while such markers were unaffected in males. Contrary, males exhibited upregulated expression of NADPH oxidase 2 and 4 (NOX2, NOX4) and inducible NO synthase (iNOS), indicating the presence of rather pro-oxidative damage.
Conclusion: Although both sexes exhibited cardiac dysfunction following AAC, males predominantly displayed a pro-oxidative cardiac environment, whereas females showed a more pronounced pro-inflammatory response. These findings enhance our understanding of sex-specific differences in pressure overload-induced cardiac injury and may contribute to the development of tailored cardioprotective therapies.

PULMONARY CONGESTION ASSESSMENT BY AUTOMATED QUANTITATIVE PHOTON--COUNTING COMPUTER TOMOGRAPHY IN HEART FAILURE PATIENTS

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Introduction: Heart failure (HF) affects interstitial lung -structure and function. AI-assisted quantitative computed tomography (qCT) offers possibility of objective assesment of lung structure, allowing to detect the presence and the severity of HF.
Study question: Can automated qCT analysis discriminate HF from controls and identify tissue structures most linked to degree of congestion?
Methods: Subjects underwent right heart catheterization (RHC), native CT chest imaging (fullinspiration/expiration) by Naeotom-α photon-counting CT scanner (Siemens), lab testing, echocardiography. Automated AI-based analysis (VIDA Diagnostics) was employed to evaluate lung volumes, densities and tissuetexture. Image series were used in mediastinal and lung window reconstruction with slice thickness 0,8 mm and increments 0,5 mm.
Results: 186 HF patients (85% HFrEF, NYHA 2.8 ± 0.5, 58 y, 80% males, NTproBNP 4491 pg/ml, LVEF 26%) and 43 controls of similar sex and BMI were analyzed; both groups free of primary lung disease. In HF, 85 % had PH (mPAP >20 mmHg). HF patients had comparable inspiratory lung volume, reduced air volume and increased lung tissue volume, compared to controls. Mean lung density (–782 ± 50 vs –832 ± 25 HU, p <0.0001), density variability and estimated lung fluid content (21.3 vs 16.7 %, p <0.0001) were all higher in HF compared to controls. Across various density cut-offs, the most notable difference was at lung tissue density < –856 HU atinspiration. Lung tissue density corelated with mean PA pressure (r = 0.49, p <0.0001), and less with pulmonary vascular resistance (r = 0.21, p = 0.006). Insp/exp scan analysis showed no difference in air trapping, but reduced lung tissue compliance (Jacobian strain: 1.8 ± 0.4 vs 1.6 ± 0.3, p = 0.0005) in HF.
Lung texture analysis showed increased ground-glass opacity (GGO) mass (48.8 g vs 9.1 g; p <0.001), reticulation (42.2 g vs. 8.4 g; p <0.001) and consolidation (0.5 g vs 0.2 g; p = 0.0011) in HF compared to controls, with no difference in honeycombing. GGO mass correlated strongly with pulmonary capillary wedge pressure (PCWP) (r = 0.48, p <0.001) and mitral regurgitation grade (r = 0.28, <0.0001), but not with inflammation (hsCRP, IL6, WBC), smoking exposure or oncotic pressure.
Conclusions: qCT distinguished HF patients from controls by increased lung tissue mass, reduced air volume, increased lung density and increased mass of GGO and reticulation. GGO mass in HF strongly reflects increased hydrostatic pressure, but not inflammation.

NEW DOUBLE-HIT RAT MODEL OF MYOCARDIAL INFARCTION WITH PULMONARY HYPERTENSION

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Introduction: Heart failure commonly leads to right ventricular (RV) dysfunction as a consequence of left ventricular (LV) dysfunction and the development of pulmonary hypertension. Although RV function is a critical prognostic factor, it has received less attention in heart failure research compared to the LV. This is partly due to the lack of robust animal models that enable the study of biventricular heart failure.
Methodology: To induce LV dysfunction, normotensive HanSD rats underwent LAD ligation in the first week. In the second week, precapillary pulmonary hypertension was induced by subcutaneous injection of VEGF

receptor blockade semaxinib (sugen, 100 mg/kg). In the seventh week, biventricular function was assessed by echocardiography, MRI, and pressure-volume analysis. Organs were weighed and RV and LV tissues collected for TaqMan PCR gene expression and histological analysis.
Results: Myocardial infarction reduced ejection fraction from 84 ± 5% to 36 ± 15% within two weeks (p <0.001), with LV wall thinning and increased internal dimensions. Infarction groups had higher heart weights, mainly due to RV hypertrophy (+70 ± 27% in IM/sugen vs sham, p <0.001). The IM/sugen group developed pulmonary congestion (+37 ± 26% lung weight, p <0.01). Cardiac output decreased in the LV of IM/placebo and in both ventricles of IM/sugen, with increased LV end-diastolic volumes. RV systolic pressure was elevated in IM/sugen (78 ± 10 mmHg, p <0.001) but unchanged in IM/placebo (40 ± 3 mmHg) compared to sham (32 ± 9 mmHg). LV contractility (end-systolic elastance and preload recruitable stroke work) declined in infarction groups, while RV systolic function (dP/dt_{max}) increased in IM/sugen (2149 ± 400 vs 1271 ± 348, p = 0.002), indicating compensated RV dysfunction, with no change in IM/placebo. We also analyzed gene expression pathways relevant to heart failure, including fibrosis (Tgfb1, Postn, Col1a1, Col3a1, Thbs4), metabolism (Acadm, Cs, Pdk4, Pgk1), inflammation (Il6, Tnf, Nox4), contractility (Myh6, Myh7, Atp2a2, Pln, Nppa), and hypertrophy signaling (Fgf23, Bmpr2, Apln, Adrb1).
Conclusion: We developed a reproducible rat model that combines myocardial infarction-induced heart failure with pulmonary hypertension. This model enables the investigation of biventricular dysfunction in heart failure, providing a platform to study the interaction between overloaded RV and LV in heart failure.

A SOLUBLE GUANYLATE CYCLASE STIMULATOR IMPROVES SURVIVAL IN A RAT MODEL OF HEART FAILURE WITH REDUCED EJECTION FRACTION AND CHRONIC KIDNEY DISEASE INDUCED BY AORTO-CAVAL FISTULA AND 5/6 NEPHRECTOMY

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Background: New therapeutic strategies for heart failure (HF) combined with chronic kidney disease (CKD) are needed, and the current knowledge, including our previous studies, suggests that targeting the NO/sGC/cGMP pathway could be a promising approach.
Purpose: We hypothesised that chronic soluble guanylate cyclase (sGC) stimulation would attenuate the course of rodent HFrEF induced by volume overload achieved by the creation of the aorto-caval fistula (ACF) combined with CKD caused by 5/6 nephrectomy (5/6 Nx).
Methods: 5/6 Nx was performed in Ren-2 transgenic rats (TGR), a model of angiotensin II-dependent hypertension, at the animal age of 8 weeks. One week later, ACF was created; another two weeks later, the animals were randomly divided into three groups, and the therapy was started: sGC stimulation with BAY-41-8543 (sGC stim.) (3 mg.kg-1. day-1 in food, n = 24), angiotensin-converting enzyme inhibitor (ACEi) (trandolapril, 2 mg.L-1 in drinking water, n = 24), or placebo (n = 22). Sham-operated TGR represented a control group (n = 8). The follow-up period was 20 weeks. GraphPad Prism 10 was used for statistical analysis with a log-rank (Mantel–Cox) test to analyse survival data.
Results: All sham-operated rats survived till the end of the study. On the contrary, all untreated HF+CKD rats died by week 12. The treatment with the sGC stim. or with ACEi similarly improved the survival rate: 46 % in sGC stim. group (p <0,0001 vs. placebo) and 58 % in the ACEi group (p <0,0001 vs. placebo).
Conclusion(s): Our results indicate that in the ACF-5/6Nx TGR animal model of combined heart failure (HF) and chronic kidney disease (CKD), treatment with an sGC stimulator reduces mortality to a similar extent as ACE inhibitor (ACEi) monotherapy compared to placebo. Further preclinical research is needed to better understand the NO/sGC/cGMP pathway in HF with concurrent CKD.

GALECTIN-3 SHAPES SYSTEMIC INFLAMMATORY SIGNATURES IN PSORIASIS: TROPONIN-RELATED SERUM CHANGES

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Introduction: Psoriasis is a chronic inflammatory skin disorder increasingly recognized as a systemic condition associated with elevated cardiovascular risk. Galectin-3 (Gal3), a α-galactoside-binding lectin involved in immune regulation and fibrosis, is implicated in the pathogenesis of both inflammatory and cardiovascular diseases. Its circulating levels are elevated in several systemic conditions, but role in modulating inflammation-related systemic changes in psoriasis remains unclear. This study aimed to investigate how Gal3 deficiency alters serum biomarker patterns and their correlation with cardiac troponin levels, as a potential surrogate marker of subclinical cardiovascular stress, in a murine model of psoriatic inflammation.
Methodology: Psoriasis-like skin inflammation was induced in C57BL/6 wild-type (WT) and Galectin-3 knockout (Gal3–/–) mice using daily topical application of Aldara cream for 7 days. Serum levels of troponin, electrolytes (Na^+, Cl^+), iron status markers (ferritin, UIBC), and complete blood count (WBC, RBC) were measured. PASI scoring was used to quantify skin inflammation. Correlation analysis was performed between troponin and other serum parameters within each group.
Results: Troponin levels did not significantly differ between groups, but distinct correlation profiles emerged. In Gal3–/– + Aldara mice, positive correlations were observed between troponin and WBC ($r = 0.71$, $p < 0.05$), PASI score ($r = 0.72$, $p < 0.05$), and UIBC ($r = 0.79$, $p < 0.05$), while RBC negatively correlated with troponin ($r = –0.71$, $p < 0.05$). In WT + Aldara mice, troponin positively correlated with Na^+ ($r = 0.76$, $p < 0.05$) and ferritin ($r = 0.83$, $p < 0.05$), while Cl^+ showed a strong inverse correlation ($r = –0.91$, $p < 0.01$). No significant correlations were found in control groups.
Conclusion: Our findings suggest that Galectin-3 modulates systemic inflammatory and hematological responses in psoriasis, as reflected through differential correlations with serum troponin. These genotype- and inflammation-dependent patterns indicate that troponin may serve as a sensitive marker for early cardiometabolic shifts even in the absence of overt myocardial injury. Further studies are warranted to explore Gal3-troponin interactions in chronic inflammation.

CHRONIC AMIODARONE TREATMENT MODIFIES ELECTROPHYSIOLOGICAL RESPONSE TO IKR BLOCK IN HUMAN FAILING VENTRICULAR MYOCARDIUM

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Introduction: Amiodarone (AMIO) is a widely used antiarrhythmic agent with proven efficacy against both ventricular and supraventricular arrhythmias. Despite its extensive clinical use, the cellular mechanisms of its antiarrhythmic action remain incompletely understood. While extracardiac toxicity is a known concern, AMIO displays relatively low proarrhythmic potential compared to other antiarrhythmic drugs.
Objective: To investigate the cellular electrophysiological effects of chronic amiodarone therapy in end-stage heart failure (HF) patients using human left ventricular tissue, and to compare responses to IKr inhibition with tissues from non-AMIO-treated HF patients and healthy donor hearts.
Methods: Left ventricular tissue slices were obtained from explanted hearts of end-stage HF patients treated or not treated with chronic AMIO, and from non-diseased donor hearts. Conventional microelectrode recordings were performed to assess action potential duration (APD), responses to 50 nM dofetilide (DOF; an IKr blocker), and the frequency dependence of the maximal upstroke velocity (V_{max}).
Results: Both AMIO- and non-AMIO-treated HF tissues exhibited significantly prolonged APD compared to donor samples (donor: 381.9 ± 13.8 ms, n = 7; HF+AMIO: 471.5 ± 27.4 ms, n = 6; HF–AMIO: 488.4 ± 3.7 ms, n = 6), but the two HF groups did not differ significantly from each other. Upon application of 50 nM DOF, donor and HF+AMIO samples showed moderate APD prolongation (donor: +21%; HF+AMIO: +37%) without early after depolarizations (EADs). In contrast, HF–AMIO samples developed EADs and failed repolarization. Furthermore, chronic AMIO treatment induced a marked use-dependent depression of V_{max}, which was absent in donor and HF–AMIO groups.
Conclusion: Chronic amiodarone therapy in heart failure patients induces a pronounced use-dependent reduction in the Vmax, a phenomenon not observed in donor hearts or in HF patients without amiodarone treatment. This distinctive electrophysiological modulation may contribute to the drug's low proarrhythmic potential and therapeutic efficacy in the failing human heart. These findings highlight a potentially protective mechanism of chronic amiodarone administration and warrant further mechanistic and clinical investigation.

HUMAN SPECIFIC CGMP REGULATION: CGMP MEASUREMENTS IN LIVING HUMAN CARDIOMYOCYTES REVEAL NEW NO-BASED DRUG TARGETS

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Cyclic guanosine 3′,5′-monophosphate (cGMP) serves as a second messenger molecule, which regulates many cel-

lular functions in health and disease. cGMP is generated by particulate or soluble guanylyl cyclases upon stimulation with natriuretic peptides or nitric oxide, respectively. Furthermore, the cGMP concentration is modulated by cGMP-degrading phosphodiesterases (PDEs). During the last decade, it has emerged that cGMP could be a novel drug target for the treatment of pulmonary and cardiovascular disorders, although its role in atrial fibrillation is rather controversial and highly unexplored. Furthermore, several new drugs currently tested in clinical trials act NO-independently with/without heme on soluble or particulate guanylyl cyclases. NO-dependent soluble guanylyl cyclases drugs are generally discarded for treatment of cardiac diseases because their effects are rather moderate or absent in mouse models, but no study has been ever performed in human atrial myocytes to directly monitor the effects of these drugs on real-time cGMP dynamics. We performed for the first time cGMP measurements in living human atrial myocytes, providing insights into cGMP functionality and signaling. Our results demonstrate that in human myocytes, cGMP levels are not only regulated by particulate guanylyl cyclases but also NO-dependent soluble guanylyl cyclase, opening up new therapeutic opportunities.

ROLE OF INFLAMMATION AND DIABETES MELLITUS IN ECHOCARDIOGRAPHIC PARAMETERS AND NEW YORK HEART ASSOCIATION FUNCTIONAL CLASSIFICATION IN PATIENTS WITH RECENT-ONSET DILATED CARDIOMYOPATHY

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Background: Dilated cardiomyopathy (DCM) is a serious disease leading to heart failure (HF). Inflammation, metabolic factors, environmental factors and aging also contribute significantly to its development. Type 2 diabetes mellitus (T2DM) is an important risk factor for a number of diseases, especially cardiovascular diseases. In the development of HF, it can affect both directly myocardial function and indirectly through comorbidities in the development of which it participates (hypertension, renal failure, etc.).
Purpose: To compare the changes of the echocardiographic (ECHO) parameters of patients with recent-onset dilated cardiomyopathy (RODCM) considering the presence of T2DM or pre-diabetes (pre-DM). The primary outcome was the change of left ventricle ejection fraction (LVEF) in 3 and 6 months after randomization and the change in New York Heart Association (NYHA) class.
Methods: Forty-eight patients were enrolled (28 men and 20 women), age from 18 to 69 years, with diagnosis of RODCM, who underwent endomyocardial biopsy (EMB). These patients were divided into two groups – with and without T2DM or pre-DM, and with or without confirmated myocarditis from EMB (>14 LCA+ and/or >7 CD3+ cells/mm^2). All patients received standard HF treatment with titration to max dose according to tolerance. Patients treated with immunosuppressive drugs were excluded, since the possibility of influencing the development or worsening of DM.
Results: Mean baseline of LVEF was for the group with T2DM or pre-DM 27.73% and 44.29% 6 months after randomization. The improvement was statistically significant (p-value <0.001). There were no patients included in the group of T2DM or pre-DM with myocardial inflammation. In the second group, including patients without T2DM/pre-DM, LVEF at the baseline was 25% in the group with myocardial inflammation and 26% in the group without myocardial inflammation. After 6 months LVEF improved to 35% in the group with myocardial inflammation and 36.75% without myocardial inflammation (*p*-value <0.001). The improvement of NYHA class was seen in both groups. However, in the subgroup with myocardial inflammation there was no improvement in the NYHA class.
Conclusion: Our findings show that statistically significant improvement of left ventricle function and functional improvement according to NYHA classification occurred in all groups. The improvement of LVEF in the group of patients with DM was significantly higher than in the group without

MONOAMINE OXIDASES LINK OXIDATIVE STRESS TO INFLAMMATION IN THE PATHOGENESIS OF VASCULAR DYSFUNCTION: INSIGHTS FROM RODENT MODELS AND HUMANS

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Oxidative stress and inflammation are interdependent pathophysiological mechanisms in the setting of cardiometabolic pathologies. Monoamine oxidases (MAO), mitochondrial enzymes with two isoforms, MAO-A and MAO-B, have emerged as important sources of reactive oxygen species (ROS) during the metabolism of catecholamines and serotonin in vascular and metabolic tissues and, more recently, as novel regulators of inflammation. In preclinical models of lipopolysaccharide-induced inflammation, we have reported an upregulation of MAO-A and MAO-B expression within the vascular wall, a process mechanistically linked to NF-κB activation. Phar-

macological inhibition of MAO significantly improved endothelium-dependent vasorelaxation and attenuated vascular hydrogen peroxide production in murine aortic rings, confirming the role of MAOs as key mediators of oxidative stress in the inflammed vasculature. In a translational approach, the MAO contribution has been further explored in mesenteric arteries and visceral adipose tissue harvested from patients undergoing elective abdominal surgery. Obese individuals exhibited markedly increased expression of MAO, accompanied by blunted vascular reactivity and enhanced ROS generation as compared to the non-obese controls. Ex vivo incubation of vascular tissues with selective MAO inhibitors alleviated endothelial function and mitigated ROS production. Furthermore, pro-inflammatory stimulation with interleukin-6 augmented MAO expression in isolated human mesenteric arterial segments, reinforcing the inflammatory regulation of MAO expression in human vascular tissue. Collectively, these data identify MAOs as inducible sources of oxidative stress in pathologies associated with chronic inflammation and underscore the relevance of MAO inhibition as a potential adjuvant therapeutic strategy for the vascular complications in the setting of cardiometabolic diseases.

EFFECTS OF DABIGATRAN AND NATTOKINASE ON *IN VIVO* AND *EX VIVO* CARDIAC FUNCTION IN ISOPROTERENOL--INDUCED HEART FAILURE RATS

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Introduction: Heart failure (HF) remains a major global health challenge, characterized by impaired cardiac function and high morbidity and mortality rates. Experimental models using isoproterenol (ISO), a synthetic β-adrenergic agonist, reliably induce cardiac injury and HF-like characteristics, providing a valuable platform to investigate potential therapeutic interventions. Dabigatran (D), a direct thrombin inhibitor, has been widely used for prevention of thromboembolism in patients with atrial fibrillation and for the treatment of venous thromboembolism. Nattokinase (NK), a fibrinolytic enzyme derived from fermented soybeans, exhibits antithrombotic and cardioprotective properties through its ability to enhance fibrinolysis and improve vascular function. This study aims to evaluate and compare the effects of D and NK on cardiac function in a rat model of ISO-induced HF, with a focus on their potential roles in mitigating cardiac dysfunction and pathological remodeling.
Methodology: Fifty male *Wistar albino* rats were randomly assigned to either a control (CTRL) group or an experimental HF (eHF) group. HF was induced in the eHF group by subcutaneous ISO administration (5 mg/kg/day) for seven consecutive days. Four weeks post-induction, the eHF group was further divided into three treatment groups: NK group (1000 Fu/kg/day), D group (15 mg/kg/day), and D+NK group (combined D and NK treatment). Treatments were administered via daily gavage for four weeks. Cardiac function was assessed through transthoracic echocardiography, and cardiodynamic parameters were evaluated in isolated hearts using the *Langendorff* technique.
Results: The combination of D and NK produced the most significant improvements in echocardiographic and cardiodynamic parameters, indicating a potential synergistic cardioprotective effect.
Conclusion: Combined administration of D and NK offers superior cardioprotective benefits in ISO-induced HF, highlighting their potential as a complementary therapeutic strategy.

CHANGE IN PULSE PRESSURE AND STROKE VOLUME VARIATION INDUCED BY PASSIVE LEG RAISE AS A PREDICTOR OF FLUID RESPONSE IN ACUTE CARDIAC CARE PATIENTS

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Introduction: Pulse pressure variation (PPV) and stroke volume variation (SVV) are established parameters to assess fluid responsiveness. These parameters are usually obtained from pulse contour analysis using hemodynamic monitors. Nevertheless, PVV and SVV values are reliable only in mechanically ventilated patients with regular sinus rhythm, therefore most of data come from perioperative care. Vast majority of intensive care unit patients doesn´t fulfill these criteria. One strategy how to use PPV and SVV in prediction of fluid response may be testing the change in PPV and SVV induced by passive leg raise (PLR). Some data suggest good reliability of SVV change after PLR in protectively ventilated surgical patients with regular sinus rhythm. Reliability of PPV and SVV change induced by PLR in non-selected intensive care population, including spontaneously breathing patients and the patients with irregular rhythm, remains unknown. Main objective of this study was to test the correlation between change in PPV and

SVV and the relative change in stroke volume following PLR.
Methodology: Consecutive patients with hypotension (systolic blood pressure <100 mmHg or on vasopressors) or signs of insufficient cardiac output (pCO_2 gap >0.8 kPa) were enrolled at cardiac intensive care unit. Hemodynamic parameters (PPV, SVV and stroke volume) were obtained from arterial pressure curve using Hemosphere hemodynamic monitor (Edwards Lifesciences, Irvine, USA) during 1-minute period immediately preceding PLR and then during 1-minute period starting 30 seconds after PLR. Mean values from these time sequences were compared.
Results: Thirty measures in 25 patients (mean age 75 ± 11 years, 22 males) were performed. Four patients were mechanically ventilated, 11 subjects had regular sinus rhythm. Significant correlation between change in both PPV and SVV and the relative change in stroke volume was observed: Spearman r = –0.51, 95% CI –0.74 to –0.18, p = 0.004 for PPV; Spearman r = –0.58, 95% CI –0.78 to –0.26, p <0.001 for SVV.
Conclusion: Change in PVV or SVV induced by PLR predicts fluid response in non-selected cardiac intensive care population.

EXPLORING THE POTENTIAL ETIOLOGICAL ROLE OF CARDIAC AUTOIMMUNITY IN POST-COVID SYNDROME

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Introduction: Persistent symptoms are frequently observed among patients recovering from COVID-19, a condition commonly referred to in the literature as post-COVID syndrome. The wide range of these complications has prompted the development of a multidisciplinary diagnostic and therapeutic approach, which has successfully identified several organ-specific components. However, the underlying pathophysiological mechanisms of post-COVID syndrome remain poorly understood. Due to the limited etiological knowledge, current therapeutic strategies are predominantly symptomatic.
Objectives: To detect autoantibodies targeting cardiac antigens in patients with post-COVID syndrome and to assess their potential clinical relevance.
Methods: Sera from 114 patients attending a post-COVID outpatient clinic were analyzed using Western blot techniques. Human cardiac tissue was homogenized, and proteins were separated by SDS-PAGE. Following transfer onto nitrocellulose membranes, the samples were incubated with patient sera. The presence of IgM and IgG autoantibodies against cardiac tissue proteins was detected using enhanced chemiluminescence (ECL).
Results: Of the 114 serum samples analyzed, 67 (59%) tested positive for cardiac autoantibodies: 19 contained IgG, 32 contained IgM, and 16 contained both IgG and IgM autoantibodies targeting cardiac proteins. Serum samples from 30 patients were also collected during a follow-up visit. In most cases, IgG or IgM autoantibodies were detected sporadically in only one of the two samples. However, persistent IgM autoantibodies were observed in some patients. The presence of cardiac autoantibodies was significantly associated with elevated C-reactive protein (CRP, p = 0.0366) and white blood cell (WBC) counts (p = 0.0387).
Discussion: A notably high prevalence of cardiac autoantibodies was observed in patients with post-COVID syndrome. The detection of persistent IgM autoantibodies may indicate impaired isotype switching or another immunological dysregulation. The association between cardiac autoantibodies and elevated inflammatory markers (CRP and WBC) suggests a potential pathophysiological link. These findings raise the possibility that cardiac autoimmunity may contribute to the multisystemic manifestations of post-COVID syndrome and may offer a target for future causal therapeutic interventions.

ASSESSMENT OF QUALITY OF LIFE IN PATIENTS WITH HFPEF USING THE EQ-5D-5L INSTRUMENT IN SERBIAN POPULATION: A CASE-CONTROL STUDY

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Introduction: Heart failure with preserved ejection fraction (HFpEF) is increasingly prevalent and significantly impacts patients' daily functioning and well-being. Evaluating health-related quality of life (HRQoL) in this population is essential for comprehensive disease management. This study aimed to assess HRQoL in patients with HFpEF compared to healthy controls using the EQ-5D-5L questionnaire.
Methodology: A case-control study was conducted at the Institute for Cardiovascular Diseases "Dedinje" from December 17, 2024, to June 11, 2025. A total of 91 participants (61 patients and 30 controls) were enrolled, including patients diagnosed with HFpEF and age-matched healthy controls. HRQoL was assessed using the EQ-5D-5L instrument, scored in accordance with the official guidebook. As no Serbian-specific value sets are available, we used Romanian value sets, per EuroQol Group guidance. The EQ-5D index and EQ-VAS (0–100 scale) scores were analyzed. Normality was assessed using Q-Q plots and histograms. Group differences were

evaluated using the Independent-Samples Mann–Whitney U test.
Results: There was no statistically significant difference in age between groups ($p = 0.138$), confirming demographic comparability. BMI, BSA, HFAPEFF score, H$_2$FPEF score, and HFpEF duration differed significantly between groups ($p < 0.001$). The EQ-5D index was significantly lower in HFpEF patients compared to controls ($p = 0.024$; U = 1181.5), and EQ-VAS scores also differed ($p = 0.001$; U = 1248.0) to the level of statistical significance, reflecting reduced self-perceived health in the HFpEF group.
Conclusion: These preliminary results suggest that the EQ-5D-5L is a promising tool for assessing HRQoL in patients with HFpEF. It is brief, easy to administer, and freely available making it suitable for both clinical and research settings in resource-limited environments. Future studies should validate EQ-5D-5L value sets for the Serbian population, further explore its utility in HFpEF cohorts, and evaluate how clinical and demographic factors influence HRQoL using multivariate analysis. Our findings support its clinical relevance and highlight the need for continued research into the impact of HFpEF on patient-reported outcomes.

INTRODUCTION OF PHOTON-COUNTING CT – DERIVED PFAI TO ASSESS CORONARY INFLAMMATION: INITIAL PRELIMINARY RESULTS

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Introduction: Chronic systemic inflammation contributes significantly to the progression of atherosclerosis. The perivascular fat attenuation index (pFAI), derived from coronary computed tomography angiography (CCTA), reflects local cytokine-driven inflammation in pericoronary adipose tissue. Our work shows inter-vessel variability in pFAI values. Using photon-counting computed tomography (PCCT), we assess pFAI in all three major coronary arteries in three distinct patient groups. No validated cut-off values currently exist for PCCT. It remains uncertain whether previously established thresholds can be directly applied, although preliminary evidence suggests similar diagnostic behavior.
Methodology: This prospective pilot study includes adults with psoriasis: (1) on ≥1 year of biologic therapy, (2) awaiting initiation of biologic therapy, and (3) healthy controls. All groups are free from known coronary artery disease. The pFAI was measured in proximal segments of the RCA, left anterior descending artery (LAD), and left circumflex artery (LCx) using Syngo.via Frontier software. Results were correlated with blood biomarkers (high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor alpha, etc.). Given the preliminary nature of our dataset, comprehensive statistical analysis is not yet feasible.
Preliminary results (n = 24): The mean pFAI values in healthy controls (n = 19) were 88.5 ± 8.0 HU (LAD), 84.0 ± 7.2 HU (LCx), and 86.1 ± 9.4 HU (RCA). Among patients receiving biologic therapy for psoriasis (n = 2), values were 92.7 ± 6.6 HU (LAD), 88.5 ± 3.3 HU (LCx), and 90.1 ± 1.2 HU (RCA). Patients with psoriasis awaiting treatment (n = 3) had values of 91.1 ± 11.4 HU (LAD), 85.1 ± 13.5 HU (LCx), and 89.9 ± 15.4 HU (RCA). Several participants exhibited pFAI values in the higher-risk range between –76.6 and –70.3 HU. Despite the absence of morphologically significant coronary plaques on CCTA, these elevated values suggest hidden cardiovascular risk.
Conclusion: This study utilizes PCCT to simultaneously assess pFAI in RCA, LAD, and LCx. Preliminary findings highlight vessel-specific variability and reveal potentially hidden cardiovascular risk in asymptomatic individuals. Expanding the sample size will be critical to determine whether chronic cytokine inhibition via biologic therapy consistently attenuates coronary inflammation across all major vessels.

STUDY OF THE EFFECT OF BETA-HYDROXYBUTYRATE ON THE CONTRACTILITY OF ISOLATED MYOCARDIUM IN RAT MODELS

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Introduction: Myocardial contractility is influenced not only by sympathetic stimulation and calcium concentration, but also by the availability of metabolic substrates. Beta-hydroxybutyrate (BOHB), a ketone body produced during fasting or catabolic states, serves as an alternative source of energy for the heart. Its increased utilization has been observed in patients with heart failure, alongside upregulation of metabolic enzymes. However, the direct inotropic effect of BOHB on myocardial tissue, particularly in diseased hearts, remains undefined.
This study aims to evaluate the effect of BOHB on the contractility of myocardial trabeculae in healthy rats and in rats with induced myocardial infarction (MI). A secondary aim was to determine whether the presence of isoprenaline, a β-adrenergic agonist, modulates the response to BOHB.
Methodology: Normotensive HanSD rats were divided into control and MI groups. MI was induced in 13-week-old males by ligation of the left anterior descending coronary artery. Seven weeks later, myocardial trabeculae were isolated and placed in an oxygenated Tyrode's solution within an organ bath. Electrical stimulation (10 V, 5 ms) was applied, and twitch force was measured at frequencies ranging from 0.5 to 5 Hz. Measurements were

taken under baseline conditions, after administration of BOHB (1 mM), and subsequently after co-administration of isoprenaline (500 nM). Anatomical characteristics of the trabeculae were measured at the end of each experiment.
Results: In healthy controls, BOHB increased twitch force across all frequencies by 47.5% (0.5 Hz), 58.8% (1 Hz), 44.9% (2 Hz), 28.8% (3 Hz), 23.8% (4 Hz) and 12.4% (5 Hz). In MI rats, the response was present but diminished: 21.2% (0.5 Hz), 38.5% (1 Hz), 31.3% (2 Hz), 14.9% (3 Hz), 7.0% (4 Hz), 11.8% (5 Hz).
The combination of BOHB and isoprenaline further amplified twitch force in both groups. In control group: 52.0% (0.5 Hz), 54.4% (1 Hz), 80.4 % (2 Hz), 77.7% (3 Hz), 78.6% (4 Hz) and 39.4% (5 Hz). In MI group: 17.6% (0.5 Hz), 39.7% (1 Hz), 44.6% (2 Hz), 36.7% (3 Hz), 16.6% (4 Hz) and 13.7% (5 Hz).
Conclusion: BOHB exhibits a clear positive inotropic effect in isolated myocardial trabeculae, which is enhanced when combined with isoprenaline. The effect is more pronounced in healthy myocardium than in infarcted tissue.

CONCOMITANT MITRAL REGURGITATION IN SEVERE AORTIC STENOSIS – INSIGHTS FROM THE CURRENT AS REGISTRY-2

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Background: Data on concomitant mitral regurgitation (MR) in patients with severe aortic stenosis (AS) are scarce.
Methods: We investigated the risk of concomitant MR in patients with severe AS in the CURRENT AS Registry-2 according to initial treatment strategy (transcatheter aortic valve implantation [TAVI], surgical aortic valve replacement [SAVR], or conservative).
Results: Among 3,365 patients with severe AS, 384 (11.4%) had moderate/severe MR (TAVI: n = 126/1,148; SAVR: n = 68/591; conservative: n = 190/1,626). The cumulative 3-year incidence of death or heart failure (HF) hospitalization was significantly higher in the moderate/severe than no/mild MR group in the entire population (54.6% vs. 34.3%, respectively; p <0.001) and for each treatment strategy (TAVI: 45.0% vs. 31.8% [p = 0.006]; SAVR: 31.9% vs. 18.7% [p <0.001]; conservative: 67.8% vs. 41.6% [p <0.001]). The higher adjusted risk of moderate/severe MR relative to no/mild MR for death or HF hospitalization was not significant in the entire population (hazard ratio [HR] 1.15; 95% confidence interval [CI] 0.95–1.39; p = 0.15); however, the risk was significant in the SAVR (HR 1.92; 95% CI 1.04–3.56; p = 0.04) and conservative (HR 1.30; 95% CI 1.02–1.67; p = 0.04) groups, but not in the TAVI group (HR 1.03; 95% CI 0.70–1.52; p = 0.86), despite no significant interaction ($p$interaction = 0.37).
Conclusions: Moderate/severe MR was associated with a higher risk of death or HF hospitalization in the initial SAVR and conservative strategies, while the association was less pronounced in the initial TAVI strategy.

PROGNOSTIC VALUE OF PULMONARY CIRCULATION PARAMETERS IN PATIENTS WITH IDIOPATHIC RECENT-ONSET DILATED CARDIOMYOPATHY

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Introduction: Heart failure (HF) is among the most important causes of morbidity and mortality in developed countries. A less documented subset of patients includes those with recent-onset dilated cardiomyopathy (RODCM), typically defined as newly diagnosed dilated cardiomyopathy (DCM) with heart failure symptoms appearing within the past six months.
Purpose: Since more extensive data about prognosis of patients with myocarditis or inflammatory cardiomyopathy are available, our study focuses purely on patients with idiopathic DCM. The study aims to evaluate pulmonary circulation parameters (PCP) obtained through cardiovascular magnetic resonance (CMR) in patients with RODCM and to assess its prognostic value in predicting improvements in the systolic and diastolic function.
Methods: Patients with a baseline CMR examination and transthoracic echocardiography (TTE) and a follow-up clinical examination with TTE performed after one year were retrospectively included. The PCP, namely pulmonary transit time (PTT), corrected pulmonary transit time (PTTc) and pulmonary transit beats (PTB), were assessed using a first-pass perfusion CMR sequence. Diastolic function was assessed with the TTE, and systolic function by both TTE and CMR.
The differences between baseline and follow-up left ventricle ejection fraction (LVEF), end-diastolic diameter (EDD), E/e´, and other parameters were calculated, and their correlations with baseline PCP were assessed.

Results: Sixty-four patients with RODCM and myocarditis excluded according to the endomyocardial biopsy, were included. On average, LVEF improved from 26.9% to 40.8%, EDD reduced from 64.6 mm to 60.8 mm and E/e´ from 12.5 to 9. PTT, PTTc, and PTB did not correlate with a change of LVEF or EDD, but all three correlated with the difference of E/e´ and diastolic function improvement assessed by TTE.
Conclusions: To our knowledge, this is the first study focused specifically on RODCM patients with EMB-excluded myocarditis and PCP as potential predictors of outcomes. Our findings show that none of the parameters correlated with the difference of the LVEF or EDD, but all PTT, PTTc, and PTB correlated with the difference of E/e´ and diastolic function improvement.

CARDIOPROTECTION VIA ADAPTATION TO CHRONIC HYPOXIA: HIF-1α AS A KEY DRIVER OF METABOLIC AND MITOCHONDRIAL REMODELING

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Adaptation to chronic hypoxia (CH) enhances myocardial tolerance to ischemia/reperfusion injury. Its cardioprotective effect is associated with the stabilization of transcription factor hypoxia-inducible factor-1α (HIF-1α), a key regulator of hypoxic response. Our previous work demonstrated that HIF-1α is essential for CH-induced cardioprotection and promotes mitophagy, a crucial event in this process. However, the underlying mechanisms remain unclear. This study aimed to elucidate the role of HIF-1α in cardioprotection induced by adaptation to CH, with a particular focus on metabolic remodeling and oxidative stress regulation.
Adult male wild-type (wt) and heterozygous Hif1a knockout (Hif1a+/–) mice were exposed to CH for four weeks or maintained under normoxic conditions. We employed liquid chromatography–mass spectrometry-based metabolomic to identify HIF-1α-dependent changes. Electron microscopy was used to assess lipid droplet abundance in left ventricular myocardial tissue. Oxidative stress was evaluated by measuring malondialdehyde (MDA) and 3-nitrotyrosine levels. In addition, mitochondrial reactive oxygen species (ROS) production was assessed using the Amplex Red assay with various substrates and inhibitors targeting different respiratory chain complexes.
Adaptation to CH induced HIF-1α-dependent metabolic remodeling and oxidative stress regulation. In wild-type mice exposed to CH, we observed significant remodeling of fatty acid composition, accompanied by increased accumulation of lipid droplets in the myocardium, indicating altered lipid storage and utilization. Adaptation of CH upregulated proteins involved in the oxidative stress response, which was reflected by reduced levels of oxidative stress markers – malondialdehyde (MDA) and 3-nitrotyrosine. In line with these findings, mitochondrial ROS production was significantly lower in the chronically hypoxic wild-type group when fatty acids were used as respiratory substrates. These protective metabolic and oxidative changes were not present in Hif1a+/– mice, highlighting the role of HIF-1α in this adaptive response.
In conclusion, our results provide novel insights into the metabolic reprogramming that occurs during adaptation to CH and underscore the central role of HIF-1α in orchestrating protective cellular responses.

HYPOXIA AND THE IMMATURE HEART

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Hypoxic states of the cardiovascular system are associated with the most frequent diseases of modern times. The degree of hypoxic injury depends not only on the intensity and duration of hypoxic stimulus but also on the level of cardiac tolerance to oxygen deprivation. This variable changes significantly during ontogenetic development. Physiological hypoxia is a normal part of fetal life for all vertebrates and plays a significant role in heart formation. However, maternal chronic hypoxia *in utero* adversely affects cardiogenesis, alters myocardial structure and induces a decline in cardiac performance. Furthermore, it may cause fetal reprogramming of several genes, which can change the susceptibility of the adult heart to oxygen deprivation; this effect is sex-dependent. Neonatal heart is highly resistant to hypoxia but the tolerance decreases after birth. These age-dependent changes are, however, not linear. The tolerance of the isolated rat heart showed a triphasic pattern: significant decrease from postnatal day 1 to 7, followed by increase in the weaning period and final decline to adulthood. The mechanisms of the high resistance of the immature heart to oxygen deprivation are not satisfactorily clarified. We have observed significant ontogenetic changes in mitochondrial oxidative phosphorylation, mitochondrial membrane potential as well as in the role of the mitochondrial permeability transition pore in the myocardial injury. These results support the hypothesis that cardiac mitochondria are deeply involved in the regulation of cardiac tolerance to hypoxia during ontogenetic development. In addition, the altered expression pattern of different cardioprotective genes likely predispose the developing heart to increased vulnerability to hypoxic injury later in life. In this connection question arises whether the already high tolerance of the neonatal mammalian heart can be further increased. It

has been found that cardioprotective mechanisms, like ischemic preconditioning or adaptation to chronic hypoxia, failed to increase hypoxic tolerance to oxygen deprivation in the highly tolerant hearts of newborn rats. It seems, therefore, that we are dealing with a common biological phenomenon: cardiac tolerance has its ceiling. These results would have important clinical implications, since cardiac sensitivity in adult patients may be significantly affected by perinatal hypoxia in a sex-dependent manner.

WHO MAY BENEFIT FROM ECMO IN CARDIOGENIC SHOCK?

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Cardiogenic shock is a life-threatening condition caused by cardiac pump failure, leading to reduced cardiac output and subsequent tissue hypoperfusion. Extracorporeal membrane oxygenation (ECMO) can rapidly restore systemic circulation and improve tissue perfusion in these patients. However, recent randomized clinical trials have not shown a clear improvement in outcomes with ECMO for cardiogenic shock.
A major limitation of these studies was the inclusion of patients who did not meet strict hemodynamic criteria for cardiogenic shock. Secondary and post-hoc analyses of these trials have highlighted specific hemodynamic parameters that may help identify patients who are more likely to benefit from ECMO. These include severely low blood pressure, low cardiac index, low mixed venous oxygen saturation (SvO_2), or a high venous-to-arterial carbon dioxide difference (pCO_2 gap).
Incorporating these findings into clinical practice could significantly refine the management algorithm for hemodynamically unstable patients with cardiogenic shock.

CONCENTRATION-DEPENDENT ELECTROPHYSIOLOGICAL EFFECTS OF AMSACRINE ON CANINE VENTRICULAR CARDIOMYOCYTES

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Amsacrine is an antineoplastic agent used in acute leukemia. QT interval prolongation, ventricular fibrillation, and sudden cardiac death have also occurred during its use. The molecule contains a methanesulfonamide group just as in some inhibitors of the rapid delayed rectifier potassium current (IKr). IKr is responsible for initiating late repolarization, its pore forming channel protein is hERG, targeted by class III antiarrhythmics. Inhibition of IKr prolongs the action potential (AP) and can cause early afterdepolarizations, increasing the risk of cardiac arrhythmias. In the case of amsacrine, it has been shown to inhibit expressed hERG channels, but native IKr and AP studies have not yet been performed, therefore we investigated the concentration-dependent effects of amsacrine on cardiomyocytes. Experiments were performed at 37 °C in enzymatically isolated canine left ventricular cardiomyocytes, which is considered a good model of the human heart. APs were measured with sharp microelectrode technique with the application of 1 and 10 μM amsacrine. Both 1 and 10 μM amsacrine caused a similar decrease in the AP Peak, the AP amplitude (APA), and the maximal rates of depolarization (V+max), and early repolarization (VPh1max). The membrane potential difference between resting value and that recorded at 20% and 50% duration of the action potential duration at 90% of repolarization (APD90), and the maximal rates of late repolarization (V-max) were all decreased. For the latter parameters, higher concentration caused larger changes. Interestingly no AP lengthening effect was observed.
Based on our results, amsacrine induced reduction of the Peak, APA, and V+max likely caused by sodium current inhibition. Reduction of VPh1max and V-max can be the result of the inhibition of transient outward and inward rectifier potassium currents, respectively. Lack of AP prolongation might be due to the simultaneous inhibition of IKr and late sodium and/or L-type calcium currents.

TO WHAT EXTENT DOES MYOSIN INHIBITION LIMIT CARDIAC PERFORMANCE?

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Direct myosin inhibitors (e.g. Aficamten) reduce left ventricular (LV) ejection fraction (LVEF) in hypertrophic cardiomyopathy (HCM) by lowering the number of force-generating myosin heads. However, their impact on subcellular determinants of contractility and global cardiac performance remains incompletely understood.
We investigated the multilevel effects of Aficamten in healthy rats, dogs, humans, and HCM patients. Global cardiovascular function was assessed by echocardiography in sedated rats. Excitation–contraction coupling was analyzed in intact LV cardiomyocytes isolated from dogs, while Ca^{2+}-sensitivity of sarcomeric force was examined in permeabilized cardiomyocytes from rats, dogs, humans,

and HCM patients at short (1.9 μm) and long (2.3 μm) sarcomere lengths (SLs).
In rats, Aficamten (2 mg/kg) reduced LVEF but preserved stroke volume (SV) and cardiac output (CO) at heart rates of 300–400 Hz, accompanied by increases in LV end-systolic and end-diastolic volumes. In intact canine cardiomyocytes, Aficamten (0.1–1 μM) concentration-dependently reduced the amplitude and duration of contraction while accelerating contraction–relaxation kinetics, without altering Ca^{2+} transient amplitude. In permeabilized cardiomyocytes, Aficamten (0.001–10 μM) decreased Ca^{2+}-sensitivity of force production across all species, yet did not impair length-dependent activation, the cellular basis of the Frank–Starling mechanism.
These findings indicate that Aficamten promotes LV diastolic filling and end-diastolic sarcomere lengthening, thereby engaging the Frank–Starling mechanism. This compensation explains the preservation of SV and CO despite reduced LVEF at clinically relevant concentrations.
Conclusion: Myosin inhibition by Aficamten limits LVEF but spares overall cardiac output through Frank–Starling–mediated compensation, providing mechanistic insight into its clinical effects in HCM.

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ELECTROPHYSIOLOGICAL EFFECTS OF EMPAGLIFLOZIN AND DAPAGLIFLOZIN: IMPLICATIONS FOR PEAK SODIUM CURRENT

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Background: Sodium-glucose cotransporter 2 (SGLT2) inhibitors have been shown to improve the clinical outcome of chronic heart failure, atrial fibrillation and reduce the risk of arrhythmias and sudden cardiac death. Despite the outstanding clinical efficacy of SGLT2 inhibitors, the underlying mechanisms for their antiarrhythmic effects are poorly understood.
Objective: We aimed to investigate the cardiac electrophysiological effect of two clinically proven cardioprotective SGLT2 inhibitors, Empagliflozin (EMPA: 1μM and 3μM) and Dapagliflozin (DAPA: 1μM and 10μM).
Methods: Action potential (AP) measurements were carried out by conventional microelectrode technique using healthy canine and failing human (explanted) ventricular preparations. The expression of Nav1.5 sodium channel in canine cardiomyocytes incubated with EMPA or DAPA for 24 hours was investigated by immunocytochemistry. To gain a deeper understanding of the electrophysiological effects of SGLT2 inhibitors, we performed computer simulations using ORd-based models.
Results: Compared to control, in the case of canine preparations, EMPA significantly (p <0.05) increased the AP amplitude (+3.3% and +5.0% n = 9) while decreased the conduction time (–5.1% and –9.8% n = 7). The maximal rate of depolarization showed a tendency to increase (n = 9), while the action potential duration remained unchanged (n = 9). In the case of failing human samples, similar tendencies were observed (n = 5). DAPA significantly increased the AP amplitude in canine preparations (n = 7) and similar tendencies were observed as in the case of EMPA in human (n = 4) and canine samples. Incubation with EMPA or DAPA for 24 hours increased Nav1.5 expression, with a more pronounced effect observed in the case of EMPA.
Conclusions: Based on our results, especially EMPA and DAPA has the potential to increase the depolarization of AP via the peak sodium current (INa) activation and upregulation of Nav1.5 expression. Theoretically, this effect could increase the impaired conduction velocity in heart failure contributing to the overall antiarrhythmic effect of SGLT2 inhibitors.

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COMPLEMENTARY CARDIOMETABOLIC EFFECTS OF EVOLOCUMAB AND SIMVASTATIN-COQ10 NANOPARTICLES IN OBESE RATS

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Introduction: Dyslipidemia, characterized by elevated LDL-cholesterol (LDL-C) and reduced HDL-cholesterol, is a major contributor to atherosclerosis and cardiometabolic diseases. Lipid-lowering agents such as statins, ezetimibe, and PCSK9 inhibitors effectively reduce LDL-C levels through distinct mechanisms. However, their clinical utility may be limited by adverse effects and suboptimal pharmacokinetics. Recent advances in targeted therapies, including nanoparticle-based drug delivery systems, aim to enhance efficacy while minimizing side effects.

Aim and methods: In this study, we investigated the pleiotropic effects of evolocumab, a monoclonal antibody PCSK9 inhibitor, and polymeric nanoparticles loaded with simvastatin and coenzyme Q10 (CoQ10), on lipid metabolism, oxidative stress, and nitric oxide (NO) signaling in obese Zucker rats, a well-established model of metabolic syndrome. Evolocumab was administered subcutaneously to obese adult male Zucker rats at 10 mg/kg every two weeks for six weeks. Concurrently, other obese Zucker rats were treated with nanoparticles loaded with simvastatin (15 mg/kg/day), CoQ10 (15 mg/kg/day), or their combination (SIMV+CoQ10) similarly for six weeks.
Results: Evolocumab significantly reduced plasma LDL-C levels and markers of lipid peroxidation, including thiobarbituric acid reactive substances and conjugated dienes, and downregulated cardiac NADPH oxidase and NF-κB expression. However, it had no effect on NO synthase (NOS) activity or endothelial NOS (eNOS) expression. In contrast, SIMV+CoQ10 nanoparticles not only decreased plasma LDL-C but also enhanced NO bioavailability via increased NOS activity and upregulation of Akt, eNOS, and phosphorylated eNOS in both the heart and aorta. Both nanoparticle-based treatments reduced NADPH oxidase and NF-κB expression, highlighting their antioxidant and anti-inflammatory potential.
In conclusion, evolocumab and SIMV+CoQ10-loaded nanoparticles both improved lipid profiles and reduced oxidative stress in obese Zucker rats. While evolocumab may protect cardiomyocytes from lipid peroxidation independently of NO signaling, only the combined nanoparticle therapy restored NO homeostasis and activated the protective Akt-eNOS pathway. These findings support the therapeutic potential of advanced lipid-lowering strategies, particularly combination and targeted approaches, in the management of cardiovascular complications in metabolic disorders.

INFECTION AND CARDIOVASCULAR DISEASE

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Infectious disease and inflammation are thought to be involved in a variety of chronic diseases including heart disease. Chlamydia Pneumoniae is typically thought to be an infectious agent for lung disease. However, it has also been associated with atherosclerotic heart disease. The talk presented in this meeting will address the questions of a direct involvement of Chlamydia Pneumoniae in cardiovascular disease. How does a lung infection cause atherosclerosis? Can Chlamydial infection actually induce atherosclerosis directly? If so, what is its mechanism of action? What factors modulate the atherogenic response in the vasculature to this infectious agent? Data will be presented using both animal models of infection and cell culture approaches to answer these important clinical questions. Ultimately, infections like Chlamydia Pneumoniae can directly induce atherogenesis but the environment plays a critical role in modulating this response.

Supported by CIHR and St. Boniface Hospital Foundation.

THERAPEUTIC POTENTIAL OF ZOFENOPRIL & DIURETICS IN PREVENTING CARDIAC REMODELING AND ISCHEMIC INJURY IN HYPERTENSION

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This study explored the cardioprotective effects of zofenopril, an ACE inhibitor, administered alone and in combination with different diuretics – hydrochlorothiazide, indapamide, and spironolactone – on ischemia–reperfusion (I/R) injury and systemic oxidative stress in spontaneously hypertensive rats (SHRs). A total of 40 male SHRs were treated orally for four weeks and divided into four groups: untreated, zofenopril monotherapy, and two combination therapy groups (ZHS: zofenopril + hydrochlorothiazide + spironolactone; ZIS: zofenopril + indapamide + spironolactone). After the treatment, we evaluated cardiac function using Langendorff's retrograde perfusion method with a 20-minute global ischemia and 30-minute reperfusion protocol. In the study we assessed the hemodynamic parameters, oxidative stress biomarkers, and histological changes in heart and kidney tissues. The applied combination therapy protocols significantly reduced both systolic and diastolic blood pressure, improved cardiac contractility and relaxation, and managed to reduce the degree of left ventricular hypertrophy. Echocardiographic data showed increased ejection

fraction and fractional shortening, particularly in the ZHS group. Although superoxide levels were reduced in all treated groups, paradoxically, other oxidative markers (H_2O_2, TBARS, NO_2^-) were elevated, and antioxidant enzyme activity (SOD) was decreased – suggesting a transient pro-oxidant adaptation phase. Our histological findings also revealed milder cardiac remodeling in treated groups, although kidney tissue showed signs of glomerular atrophy and tubular damage, especially in the combination therapy groups.
In conclusion, zofenopril-based combination therapies offer meaningful cardiovascular protection in hypertensive rats by increasing myocardial performance and mitigating structural damage during I/R injury. However, since the antihypertensive treatment was relatively short (only 4 weeks), in order to elucidate or deny the prooxidative mechanism, additional studies over longer time intervals would be needed.

SERUM NEPRILYSIN ACTIVITY ASSAY AND ITS IMPLICATIONS IN RENAL AND ONCOLOGIC CONDITIONS: A CARDIOVASCULAR PERSPECTIVE

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Neprilysin (NEP) is a zinc-dependent metallopeptidase involved in the degradation of several vasoactive peptides, including natriuretic peptides, which play key roles in cardiovascular homeostasis. Dysregulation of NEP function may therefore contribute to cardiovascular impairment, particularly in conditions such as chronic kidney disease (CKD) and malignancy. Therefore our objective was to develop an analytical method that enables efficient measurement of soluble NEP activity, paving the way for its potential diagnostic application.
NEP concentration in human serum was measured using a commercially available ELISA kit, and NEP enzymatic activity was quantified using a newly developed chromogenic assay. Serum samples from 125 individuals were analyzed, including 109 non-tumor controls, 7 patients with advanced CKD, and 9 patients with active malignant disease.
No direct correlation was observed between NEP concentrations and activities (r = 0.01722), which we attributed to peripheral degradation of NEP. Concentrations did not show significant differences between patients with tumors and those without tumors (median = 3896 ng/L, [min–max: 164.2–110960 ng/L]; n = 107 vs. median = 5118 ng/L, [min–max: 310.9–23981 ng/L]; n = 9; p = 0.8555). Similarly, samples from patients with CKD showed no significant difference in NEP concentration compared to non-tumor control individuals (median = 3395 ng/L, [min–max: 1753–111966 ng/L]; n = 7; p = 0.1242). In contrast, the soluble NEP activity measured with our optimized method was found to be significantly higher in samples from oncology patients (median = 82.12 U/L [min–max: 32.44–280.6 U/L]; n = 9) compared to non-tumor controls (median = 7.95 U/L [min–max: 1.62–42.09 U/L]; n = 107; p <0.0001). Likewise, the CKD group exhibited elevated CD10 activity (median = 36.50 U/L [min–max: 8.40–167 U/L]; n = 7; p <0.0001).
These findings indicate that while NEP concentration as measured by immunoassay does not differ significantly across clinical groups. NEP enzymatic activity is elevated in CKD and cancer. From a cardiovascular perspective, increased NEP activity may enhance the degradation of natriuretic peptides, reducing their protective effects and possibly contributing to cardiovascular complications. The chromogenic NEP activity assay presented here may serve as a useful biomarker tool for identifying individuals at elevated cardiovascular risk and supports further exploration of NEP-targeted strategies in cardio-oncology and nephrology.

CIEDS IN PATIENTS WITH CARDIAC SARCOIDOSIS – DATA FROM CLINICAL PRACTICE

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Introduction: Sarcoidosis is an idiopathic multisystem granulomatous disease of unknown etiology. In the Czech Republic the incidence is more than 3 per 100,000 inhabitants. Approximately 5 % of cases involve myocardial infiltration. The literature on the subject indicates that the disease manifests through various degrees of atrio-ventricular (AV) blocks, as well as ventricular tachycardias and, in extreme cases, sudden cardiac death. The indications for CIED (cardiac implantable electronic device) implantation and the subsequent therapeutic interventions are subjects that require further discussion.
Method: Retrospective analysis of a registry of consecutive patients diagnosed with cardiac sarcoidosis in a single center.
Results: Between 2013 and 2024, definite cardiac sarcoidosis was diagnosed in 13 patients (median age 54 years, range 45 years-65 years, 8 women). Diagnosis remains challenging due to the variable presentation and the patchy nature of the infiltration. Traditionally relies on histological identification but sensitivity is limited and non-invasive methods as FDG-PET-CT and cardiac MRI are emerging as a main method these days. It is not uncommon for methods to be repeated.

The following diagnostic procedures were performed: 12 FDG-PET-CT scans (10 positive, 2 false negative), 7 cardiac MRI scans (5 positive, 2 false negative), 4 endomyocardial biopsies (1 false negative result) and 9 bronchoscopies with biopsy (1 false negative result).
Implantation of cardiac implantable electronic devices (CIEDs) was performed on 13 patients (100% of those with a cardiac form). Of these, 11 patients had devices with defibrillator function (ICD or CRT-D), while two patients had only a pacemaker. The analysis revealed a total of 10 patients with bradyarrhythmia, the most prevalent being grade 3 AV block in 6 patients.
During the follow-up, a total of 12 patients were documented to have ventricular tachycardia (VT), including 7 patients with non-sustained VT, 5 patients with sustained VT, 2 patients with an electrical storm, and 2 patients with ventricular fibrillation. Catheter ablation was performed in 4 patients.
Conclusion: The prevalence of rhythm disturbances is high in cases of cardiac sarcoidosis. The most common bradyarrhythmia are AV blocks of grade 3. In young patients with higher-grade AV block, a thorough investigation of the possible etiology is necessary. Tachycardia complications that require further management are frequent.

EXERCISE-INDUCED DESATURATION IN PATENT FORAMEN OVALE: INSIGHTS FROM THE MEASURE PFO STUDY

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Introduction: Exercise-induced desaturation is a rare manifestation of patent foramen ovale (PFO), caused by exertion-related right-to-left (R-L) shunting. The exact mechanisms are not fully understood. Intense physical activity may shift thoracic structures and increase venous return, raising right atrial (RA) pressure. This can transiently reverse the usual left-to-right atrial pressure gradient, directing deoxygenated blood through the PFO and bypassing pulmonary circulation. These events are likely inducible, not persistent, and underrecognized despite PFO occurring in up to 25% of the population.
Methodology: Twelve patients with ischemic stroke or TIA and confirmed PFO underwent cardiopulmonary exercise testing (CPET) before and after percutaneous closure. Oxygen saturation was measured by forehead reflectance oximetry. In all, oxygen saturation progressively dropped down during CPET upon exertion before closure. Spirometry was normal. R-L shunt was quantified by catheter-based thermodilution (Inntherm®) at rest and during Valsalva maneuver. PFO closure was uncomplicated, followed by repeated CPET.
Results: Ten of twelve patients were men; mean age 48 ± 7 years, BMI 29 ± 4. Hypertension affected 42%, diabetes 17%, and hyperlipidemia 58%. Smoking status: seven non-smokers, three smokers, two ex-smokers. Hemoglobin averaged 149 ± 10 g/L. Pre-closure VO_{2max} was 31 ± 6.4 mL/kg/min, max. workload 246 ± 60.5 W, post-exercise SpO_2 90.8 ± 2.6%. PFO tunnel length was 12.7 ± 2.4 mm, height 4.7 ± 1 mm. Aneurysmal interatrial septum was present in 67%, eustachian valve in 42%. Mean RA pressure was 6 ± 2 mmHg, LA pressure 10 ± 5 mmHg. Bubble test showed grade 3 (>25 bubbles) in 6 and grade 4 (opacification of left atrium) in 5 patients. Provoked shunt during Valsalva averaged 0.96 ± 0.9 L/min. One patient received an 18mm left atrial disc occluder; all others received larger devices, mostly 25mm occluders. Post-closure VO_{2max} was 32 ± 8.1, workload 251 ± 63.8 W, and SpO_2 improved to 95 ± 3% (p <0.001).
Conclusion: Provoked exercise desaturation due to PFO resolved fully after closure, with no loss of exercise performance; two patients reported subjective improvement. The significant increase in post-exertional oxygenation supports transient R-L shunting as the cause. PFO should be considered in differential diagnosis of exertional hypoxemia. While CPET is not suitable for screening, percutaneous closure is a safe and effective treatment in selected patients.

NEW ONSET OF ARRHYTHMIA AFTER PATENT FORAMEN OVALE (PFO) CLOSURE STUDY – PRELIMINARY RESULTS

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Introduction: Percutaneous patent foramen ovale (PFO) closure is an established intervention for secondary prevention of embolic stroke of undetermined source (ESUS). However, new-onset atrial fibrillation (AF) – typically transient and occurring within 45 days—is a recognized post-procedure complication. Reported incidence varies widely (2.8–37% in high-monitoring studies, ~5% in meta-analyses) and is likely underestimated. Proposed mechanisms include device-induced irritation and localized inflammation. Although the associated stroke risk appears low, the need for long-term anticoagulation remains uncertain.
Methodology: This ongoing prospective, single-centre study (ClinicalTrials.gov ID: NCT06983613) at Na Homolce Hospital enrols ESUS patients undergoing PFO closure. Participants undergo extended ECG Holter monitoring (three weeks: days 7–28 post-procedure; one week at one year). The primary endpoint is atrial arrhythmia (AF, atrial flutter, or atrial tachycardia) lasting ≥30 seconds; the secondary endpoint is identification of associated risk factors. Inclusion criteria include: age >18 years; diagnosis of stroke with an indication for percutaneous PFO closure

confirmed by interdisciplinary cardiology-neurology consensus, per current guidelines; and written informed consent. Exclusion criteria include any known history of atrial arrhythmia, screened by Holter monitoring. The study began in December 2024.
Results: Among 28 patients who completed monitoring (mean age 54 ± 12 years, 95% CI: 49–59 years; 54% female), new-onset atrial arrhythmia was detected in 6 (21.4%). All experienced AF; one also had atrial tachycardia. Episodes occurred between days 3 and 17 post-closure (mean onset 10 ± 6 days) and lasted from 5 minutes to 2 days and 19 hours. Each patient experienced multiple paroxysms (range: 2–19). Four men and two women were affected, with a mean age of 55 years. The longest single episode occurred in a 70-year-old male. Additional patients are currently undergoing ECG monitoring. Three participants who initially consented later declined monitoring.
Conclusion: Preliminary data suggest a 21.4% incidence of early atrial arrhythmia following PFO closure, supporting concerns of underrecognition. These findings underscore the need for further investigation into incidence, risk factors, stroke recurrence implications, and long-term anticoagulation strategies. This study aims to inform optimal patient selection, procedural planning, and post-closure care.

LABORATORY AND CLINICAL DETERMINANTS OF ELIGIBILITY FOR IRON REPLACEMENT IN HEART FAILURE PATIENTS WITH REDUCED EJECTION FRACTION: A RETROSPECTIVE COHORT STUDY

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Background and aim: Iron deficiency (ID) is a common comorbidity in patients with heart failure with reduced ejection fraction (HFrEF) and adversely affects functional status and prognosis. The ESC 2021 guidelines recommend intravenous iron replacement in patients with HFrEF meeting laboratory criteria: ferritin < 100 µg/L or ferritin 100–300 µg/L with TSAT <20% and HGB ≤150 g/L (class IIa/B).
This retrospective study analyzed the reasons for not meeting the criteria and the relationship between laboratory and clinical parameters.
Methods: 176 patients with HFrEF (EF <45%) who were followed up at a tertiary cardiology center were included in the study. Laboratory parameters (HGB, ferritin, TSAT), clinical characteristics (NYHA, NTproBNP, BMI) and therapy (anticoagulation, antiplatelet therapy) were evaluated.
Comparisons between groups meeting and not meeting criteria were performed using chi-square tests and t-tests.
Results: 98 (55.7%) of 176 patients met the criteria for iron replacement.The main reasons for non-compliance included: HGB >150 g/L (40%), TSAT ≥20% with ferritin 100–300 µg/L (45%) and ferritin > 300 µg/L (15%). BMI and NTproBNP parameters were not significantly associated with meeting the criteria (p >0.05).
Conclusion: HGB >150 g/L was a major barrier to iron replacement, suggesting the need to re-evaluate the thresholds in the ESC guidelines. The results highlight the importance of individualizing therapy based on clinical symptomatology.

THE CLINICAL COURSE OF PHENOTYPE--NEGATIVE PATIENTS WITH THE POSITIVE GENOTYPE OF HYPERTROPHIC CARDIOMYOPATHY

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Background: Hypertrophic cardiomyopathy (HCM) is a genetic myocardial disease. In 20–30% of patients, a disease-causing mutation is identified and may also be present in relatives. Individuals with a confirmed mutation but no left ventricular hypertrophy (LVH) are classified as genotype-positive, phenotype-negative (G+/P–). The clinical course of these individuals, particularly in terms of LVH development and adverse HCM-related outcomes, remains unclear.
Purpose: To describe the clinical course of G+/P- individuals under regular follow-up at a nationwide tertiary center.
Methods: G+/P– individuals were recruited from relatives of HCM patients. Evaluations included electrocardiography (ECG) and transthoracic echocardiography (TTE). A phenotype-negative status was defined by maximal left ventricular wall thickness (MLVWT) <13 mm. HCM was diagnosed if MLVWT ≥13 mm developed during follow-up without a causal haemodynamic factor. Genetic testing was performed via next generation sequencing; mutations were classified by ACMG/AMP guidelines. Individuals with likely pathogenic (LP) or pathogenic (P) mutations were considered genotype-positive.
Results: Thirty-four individuals were identified as G+/P–, with a mean age of 31.7 ± 14.8 years; 27% were male. The most frequent mutations were in MYBPC3 (77%) and MYH7 (24%). At baseline, 71% had normal ECGs, and most had no comorbidities. Eighty-five percent were asymptomatic, and 9% reported NYHA class II dyspnea. Mean follow-up was 6.3 ± 3.8 years, with complete ECG and TTE data available for 79%. Over time, MLVWT in-

creased from 9.6 ± 1.6 mm to 10.6 ± 2.5 mm (p = 0.01), while LV end-diastolic diameter and ejection fraction remained unchanged (p = 0.23 and p = 0.21). The percentage with a normal ECG remained stable (71% vs. 70%, p = 0.56). LVH developed in six individuals (18%). Mean time to HCM diagnosis was 4.8±4.5 years. No G+/P– individual with new HCM experienced major HCM-related adverse events. One patient with LVH had non-sustained ventricular tachycardia (nsVT) on Holter ECG; following risk stratification, an implantable cardioverter defibrillator (ICD) was placed.
Conclusion: G+/P– individuals were young with few symptoms or comorbidities. Eighteen percent developed HCM during follow-up. One patient received a primary prevention ICD. These findings support ongoing TTE and ECG monitoring in G+/P– individuals.

THE ANALYSIS OF NECROPTOTIC AND AUTOPHAGIC SIGNALING PATHWAYS IN CARDIOMEGALY AND THE POTENTIAL ROLE OF BIOLOGICAL SEX

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Introduction: Necroptosis, a regulated necrotic cell death, is involved in various cardiovascular pathologies. Its relevance and interplay with autophagy in cardiomegaly, along with sex differences, remain unclear.
Methodology: Male and female Wistar rats underwent abdominal aortic constriction (AAC) at the second postnatal day to induce cardiomegaly. After 90 days, the rats were euthanized, and the left ventricular tissue was used for molecular analyses via SDS-PAGE/Western Blotting.
Results: The left and right ventricle to total body weight ratios were significantly higher in both sexes with cardiomegaly, while lung/body weight ratio was elevated only in males. Necroptosis was confirmed in both sexes by increased phosphorylated forms of its key proteins, receptor-interacting protein kinase 3 (RIP3) and mixed lineage kinase domain-like protein (MLKL). The levels of autophagic regulators, including pSer555-ULK1, Beclin-1, LC3-I and -II, and mTOR, were significantly increased in male cardiomegalic rats. In contrast, phosphorylated AMP-activated protein kinase (pAMPK), was elevated only in females with cardiomegaly.
Conclusion: This study demonstrated a potential role of necroptosis in cardiomegaly in both males and females, while autophagy dysregulation likely occurs only in males. This may indicate the need for individualized pharmacological approaches, but further research is necessary to explore other potential sex-specific influences.

PREVALENCE AND PROGNOSIS OF MULTIMORBIDITY IN HEART FAILURE WITH MILDLY REDUCED EJECTION FRACTION

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Objective: The study investigates the prevalence and prognostic impact of multimorbidity in patients hospitalized with heart failure (HF) with mildly reduced ejection fraction (HFmrEF).
Background: Related to ongoing demographic changes and the ageing population, the number of patients with cardiac and non-cardiac comorbidities and specifically the proportion of patients with multimorbidity increases in patients with HF.
Methods: Consecutive patients hospitalized with HFmrEF were retrospectively included at one institution from 2016 to 2022. Patients were divided into four groups (i.e. 0–1, 2–3, 4–5, ≥6 comorbidities) based on the number of concomitant comorbidities taking into account a total of 12 comorbidities (i.e., valvular heart disease, arterial hypertension, atrial fibrillation/flutter, prior/current acute myocardial infarction, stroke, peripheral artery disease, anemia, diabetes mellitus, chronic kidney disease, malignancy, liver cirrhosis, and chronic obstructive pulmonary disease). The prognostic impact of the number of comorbidities was investigated with regard to the primary endpoint of all-cause mortality at 30 months (median follow-up).
Results: From a total of 2,184 patients hospitalized with HFmrEF, 37% presented with 4–5 and 17% with ≥6 comorbidities. The most common comorbidities were arterial hypertension (78%), anemia (51%) and atrial fibrillation/flutter (44%). Compared to patients with 4–5, 2–3, 0–1 comorbidities patients with ≥6 comorbidities were more likely to be discharged with beta-blockers (83.6% vs. 78.7% vs. 77.6% vs. 64.7%; p = 0.001), loop diuretics (83.3% vs. 58.6% vs. 32.3% vs. 14.9%; p = 0.001) and mineralocorticoid receptor antagonists (MRA) (17.2% vs. 15.7% vs. 12.8% vs. 7.9%; p = 0.004). The risk of all-cause mortality at 30 months increased with the number of comorbidities and was higher in patients with ≥6 comor-

bidities compared to patients with less (i.e., 4–5, 2–3, 0–1) comorbidities (59.5% vs. 38.6% vs. 17.0% vs. 7.9%, p = 0.001). Accordingly, an increasing number of comorbidities was associated with a higher risk for rehospitalization due to HF worsening at 30 months (29.0% vs. 14.9% vs. 7.5% vs. 2.9%, p = 0.001), which was still evident after multivariable adjustment. Finally, both cardiovascular (adjusted HR = 1.106; 95% CI 1.030–1.188; p= 0.006) and non-cardiovascular (adjusted HR = 1.564; 95% CI 1.470–1.664; p = 0.001) comorbidities predicted the risk of long-term all-cause mortality.
Conclusion: In patients hospitalized with HFmrEF, more than 50% presented with at least 4 comorbidities. Both cardiovascular and non-cardiovascular comorbidities predicted the risk of long-term all-cause mortality in HFmrEF.

DISTINCT EFFECTS OF PDE5 INHIBITION ON CGMP AND CALCIUM SIGNALLING IN HUMAN ATRIAL MYOCYTES FROM PATIENTS IN SINUS RHYTHM VERSUS ATRIAL FIBRILLATION

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Background: Cyclic guanosine monophosphate (cGMP) is a ubiquitous second messenger present in many tissues. Its signaling pathway is regulated by phosphodiesterases (PDEs), such as PDE5. While the role of PDE5 has been extensively studied in various tissues and models, its function in the human heart remains unexplored. Recent studies have reported protective effects of PDE5 inhibitors, but there have been conflicting reports of episodes of atrial fibrillation (AF) following the use of sildenafil, a PDE5 inhibitor used to treat erectile dysfunction. The aim of this study was to determine how PDE5 regulates cGMP levels in human atrial myocytes and how this regulation affects atrial cellular electrophysiology with a special focus on AF.
Materials and methods: Human atrial myocytes were isolated from atrial tissue of patients in sinus rhythm (SR) or with AF. Cells were transduced with adenoviral vectors expressing FRET-based biosensors to monitor real-time changes in cGMP and cAMP. Voltage-clamp experiments were performed to assess L-type Ca^{2+} current, sarcoplasmic reticulum Ca^{2+} load, and spontaneous transient inward currents (ITi). Confocal Ca^{2+} imaging was employed to evaluate Ca^{2+} sparks and waves. Sildenafil (1 μM) was used to inhibit PDE5 and IBMX (100 μM) as a non-selective PDE inhibitor.
Results: PDE5 inhibition induced an increase in cGMP levels in both groups, however, this increase was lower in cells from AF patients. In myocytes from patients in SR, PDE5 inhibition might be associated with antiarrhythmic effects, such as small reduction in Ca^{2+} sparks and waves, Iti frequency, and sarcoplasmic Ca^{2+} content. In contrast, sildenafil elicited proarrhythmic responses in the cells obtained from patients with AF, enhancing spontaneous Ca^{2+} release events and Iti frequency, probably due to a PDE crosstalk which enhances cAMP levels upon PDE5 stimulation in cells from these patients.
Conclusion: These findings reveal that AF-associated molecular remodeling includes alterations affecting PDE5 and cGMP signaling in human atrial myocytes. The differential responses to PDE5 inhibition between SR and AF patients provide a mechanistic explanation for the contradictory observations. These findings also highlight the importance of re-evaluating the safety and efficacy of PDE5 inhibitors as potential antiarrhythmic agents.

CARDIO-ONCOLOGIC CONSIDERATIONS IN HEART FAILURE WITH MILDLY REDUCED EJECTION FRACTION: PREVALENCE AND PROGNOSTIC IMPACT OF MALIGNANCIES

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Objective: The study sought to assess the prevalence and prognostic impact of malignancies in patients with heart failure and mildly reduced ejection fraction (HFmrEF).
Background: Patients with malignancies were commonly excluded from prior heart failure (HF) trials resulting into limited data regarding the outcomes of HF patients with concomitant malignancies.
Methods: Consecutive patients hospitalized with HFmrEF were retrospectively included at one institution from 2016 to 2022. The prognosis of patients with malignancies was investigated regarding the primary endpoint all-cause mortality at 30 months (median follow-up). The key secondary endpoint was rehospitalization for worsening HF.

Results: Among 2,184 patients with HFmrEF, 15.3% (n = 335) suffered from concomitant malignancies (prior: n = 78; active: n = 257). In patients with active malignancies, most patients had solid (n = 175) followed by hematological malignancies (n = 73) (both: n = 9). Patients with malignancies had lower rates of ischemic cardiomyopathy, less frequently underwent invasive coronary angiography and were less commonly treated with beta-blockers and statins. Patients with malignancies were accompanied by a higher risk of long-term all-cause mortality (59.4% vs. 26.2%; p = 0.001), which was already observed during index hospitalization (8.4% vs. 2.5%; p = 0.001). After multivariable adjustments, malignancies were identified as independent predictor of all-cause mortality at 30 months (adjusted HR = 2.614; 95% CI 2.166–3.156; p = 0.001), but not HF-related rehospitalization (adjusted HR = 0.723; 95% CI 0.487–1.074; p = 0.108). The prognosis of patients with solid and hematological malignancies was comparable (adjusted HR = 0.797; 95% CI 0.555–1.145; p = 0.219).
Conclusion: Malignancies were present in 15% of patients with HFmrEF and identified as strongest predictor of long-term all-cause mortality.

NATURAL CARDIOPROTECTION IN PSORIASIS: EFFECTS OF *MELISSA OFFICINALIS* EXTRACT ON HEART PERFORMANCE AND OXIDATIVE MARKERS

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Psoriasis is increasingly recognized as a systemic immune-mediated disorder that may impair cardiac function. Emerging evidence suggests that certain herbal therapies could mitigate the cardiovascular effects associated with psoriasis. This study aimed to evaluate the cardioprotective potential of *Melissa officinalis* (MO) extract in an isolated heart model using psoriatic rats. A total of 24 male Wistar albino rats were assigned to three groups: control (CTRL), psoriasis (PSORI), and psoriasis treated with *M. officinalis* extract (PSORI+MO). Psoriasis was induced by daily topical application of 5% imiquimod cream for seven consecutive days. Following induction, rats in the PSORI+MO group received *M. officinalis* extract orally (200 mg/kg b.w.) for three weeks. The Psoriasis Area and Severity Index (PASI) was recorded after induction and weekly throughout the treatment period. Cardiac function was assessed using the Langendorff perfusion system, measuring dp/dt max and min, systolic and diastolic left ventricular pressure (SLVP, DLVP), heart rate (HR), and coronary flow. Oxidative stress parameters, including TBARS, NO, O_2, and H_2O_2, were determined in coronary venous effluent. Treatment with *M. officinalis* significantly reduced erythema and scaling in psoriatic rats. Psoriasis was associated with impaired cardiac contractility and relaxation, along with elevated SLVP and DLVP. Administration of *M. officinalis* improved cardiac performance and reduced both SLVP and DLVP. Additionally, a marked decrease in oxidative stress biomarkers was observed in the PSORI+MO group compared to psoriatic rats. *Melissa officinalis* extract exerted beneficial effects on both skin symptoms and cardiac function, while also modulating oxidative stress in the hearts of psoriatic rats.

THE POTENTIAL OF MOLECULAR HYDROGEN IN THE TREATMENT OF FAILING MYOCARDIUM AND OTHER CARDIOVASCULAR PATHOLOGIES

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Introduction: Heart failure (HF) is a clinical syndrome arising from structural or functional abnormalities of the heart, leading to reduced cardiac output and a variety of related symptoms. Despite advances in treatment and medical devices, HF remains associated with high morbidity and mortality, ranking among the leading causes of death worldwide. In recent years, molecular hydrogen (H_2) has emerged as a promising cardioprotective agent, boasting an excellent safety profile across various routes of administration and an increasing body of evidence supporting its clinical benefits. This presentation focuses on the potential adjuvant applications of H_2 in cardiovascular medicine in light of the latest clinical data and numerous positive effects.
Methods: We evaluated 61 registered clinical trials and 34 published human studies devoted to the use of H_2 in cardiovascular medicine. Three main modes of administration were examined: inhalation of H_2 (1–4%), drinking hydrogen-enriched water (1–1,5 liters per day), and intravenous infusion of hydrogen-rich saline.
Main findings: Clinical evidence demonstrates a significant therapeutic potential of H_2 for several cardiovascular diseases. In heart failure, H_2 improved left ventricular ejection fraction and reduced BNP levels through modulation of the p53 pathway. In the largest hypertension study, a significant reduction in blood pressure (–7.81/–2.89 mmHg) was observed when H_2 was used in combination with standard therapy. Based on study results, H_2 acts mechanistically as a selective antioxidant, exhibits anti-inflammatory effects, and protects mitochondria without disrupting physiological redox signaling.

Future directions: H_2 therapy represents a paradigm shift towards multi-targeted interventions in cardiology with an exceptional safety profile. Potential future applications include personalized dosing guided by oxidative stress biomarkers and combined therapies with existing medications. The synergy of strong preclinical research and favorable clinical outcomes could enable the broad integration of H_2 into cardiology practice.
Conclusion: Molecular hydrogen is an innovative, safe, versatile, and potentially breakthrough option for the treatment of cardiovascular diseases. Demonstrated efficacy in heart failure, hypertension, and acute coronary syndromes confirms its ability to target key disease mechanisms while maintaining excellent tolerability. As clinical evidence grows and regulatory frameworks evolve, H_2 has the potential to become a standard component of cardiological therapy.

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CONVENTIONAL VS. OPTIMIZED PERIPROCEDURAL ANALGOSEDATION VS. TOTAL INTRAVENOUS ANESTHESIA FOR PULSED-FIELD ABLATION: A RANDOMIZED CONTROLLED TRIAL (COOPERATIVE-PFA)

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Introduction: Deep analgosedation (DAS) or general anesthesia (GA) is mandatory for pulsed-field ablation (PFA) of atrial fibrillation (AF). In contrast to DAS, GA (conventional or total intravenous anesthesia [TIVA]) requires airway management. To find the optimal sedation regimen, this study compared ketamine-remimazolam DAS and propofol-opioid TIVA to propofol-opioid DAS, focusing on sedation-related adverse events.
Methods: Patients indicated for AF catheter ablation were randomly assigned in a 1 : 1 : 1 ratio to (1) DAS using intermittent propofol-opioid boluses (arm P), (2) continuous remimazolam-ketamine DAS (arm R), or (3) continuous propofol-opioid TIVA with secured airways (arm TIVA). Catheter ablation was performed using the FARAPULSE system (Boston Scientific, MA, USA). The major exclusion criterion was obstructive sleep apnea syndrome. The primary endpoint was defined as a composite of hypoxemia, hypotensive, or hypertensive events requiring intervention or leading to procedure discontinuation. Secondary endpoints included hemodynamic instability events, procedure time, serious adverse events, and patient satisfaction.
Results: One-hundred and twenty-seven patients (mean age 62.9 ± 10.3 years, 35.1% female, 47.2% with paroxysmal AF) were enrolled and randomized to the P (N = 42), R (N = 43) or TIVA (N = 42) arms. The primary endpoint occurred in 85.7% of P pts., 27.9% of R pts., and 66.7% of TIVA pts. (p <0.001), driven by hypoxemia in the P arm (100% of pts. with the primary endpoint) and by hypotension in the TIVA arm (100%). The R arm showed a similar distribution of hypoxemia (50%) and hypotensive (66.7%) events. No differences were observed in mean procedural times, rates of serious adverse events, and assessment of patient satisfaction.
Conclusions: In PFA procedures for AF, remimazolam-ketamine DAS was superior to propofol-opioid regimens (either boluses or continuous) and had the lowest risk of hypoxemia and hypotensive events. More than 80% of patients undergoing conventional propofol-opioid analgosedation experienced hypoxemia.

EFFECTS OF IMEGLIMIN ON PLATELET MITOCHONDRIAL RESPIRATION AND OXIDATIVE STRESS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Introduction: Imeglimin is a recently approved antidiabetic drug with a unique mode of action that targets mitochondrial dysfunction- an essential factor in the pathogenesis of type 2 diabetes mellitus (T2DM). Previous cellular studies have shown that imeglimin improves mitochondrial function and reduces oxidative stress. Based on the premise that platelet mitochondrial respiration serves as a biomarker of systemic bioenergetic dysfunction, we aimed to investigate whether imeglimin exerts similar effects on mitochondrial respiration in platelets sampled from patients diagnosed with T2DM.
Methodology: Peripheral venous blood was sampled from 15 patients with confirmed T2DM. Platelets were isolated in accordance with a standardized protocol and incubated with increasing concentrations of imeglimin (1500 µg/mL,

3000 µg/mL, 6000 µg/mL). High-resolution respirometry was performed at 37 °C using the Oxygraph-2k (Oroboros Instruments, Austria). In platelets permeabilized with digitonin, mitochondrial respiratory parameters were studied: OXPHOS capacity (maximal active respiration), LEAK (non-phosphorylating respiration), and ET capacity (maximal uncoupled respiration), all corrected for residual oxygen consumption (ROX). Oxidative stress was quantified using the Ferrous Oxidation–Xylenol Orange (FOX) assay.
Results: Imeglimin did not induce significant changes in mitochondrial respiratory parameters. However, a notable and consistent reduction in ROX was observed with increasing imeglimin doses. FOX assay results showed a significant, dose-dependent decrease in hydrogen peroxide levels, indicating lower oxidative stress.
Conclusions: In patients with T2DM, although imeglimin did not alter key mitochondrial respiratory parameters in platelets, it significantly reduced oxidative stress in a concentration-dependent manner.

PYRIDOSTIGMINE TREATMENT SIGNIFICANTLY ALLEVIATES ISOPRENALINE-INDUCED CHRONIC HEART FAILURE IN RATS

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Background: Autonomic imbalance is one of the major pathological disturbances in chronic heart failure (CHF). A growing body of evidence suggests cholinergic potentiation as a potential therapeutic approach in CHF, since it corrects the autonomic imbalance and alters the inflammatory response via the cholinergic anti-inflammatory pathway. However, there is a gap in knowledge regarding different cholinergic potentiation methods and their specific mechanisms of action.
Methodology: CHF was induced in male Wistar albino rats using an isoprenaline model (5 mg/kg/day s.c. for 7 days, followed by 4 weeks of CHF development). Afterwards, rats received pyridostigmine (22 mg/kg/day in tap water for 14 days) or no treatment. Electrocardiographic and echosonographic measurements were obtained, and samples were taken for antioxidative status, NTproBNP, MMP2, and MMP9 measurement and histopathological analysis.
Results: Pyridostigmine treatment significantly attenuated the progression of CHF, preventing further dilatation of the left ventricle (Iso LVIDd, LVIDs, EDV, and ESV $p < 0.001$ versus Iso + Pyr) and systolic dysfunction (Iso EF $p < 0.001$ versus Iso + Pyr). The further thinning of the ventricle wall was also diminished by pyridostigmine administration. Additionally, pyridostigmine improved antioxidative status (↓ TBARS, ↓ $NO2^-$; ↑ CAT, ↑ GSH) and significantly reduced cardiac fibrosis development, confirmed by pathohistological findings and biochemical marker reduction (↓ MMP2, ↓ MMP9).
Conclusion: In the present study, subacute peripheral AChE inhibition via pyridostigmine showed compelling antioxidative, anti-inflammatory, and antifibrotic effects, with the subsequent preservation of cardiac functional parameters in a rat model of CHF. However, further investigations are needed to fully understand the exact cellular mechanisms involved in the CHF attenuation via pyridostigmine.

CHRONIC SALT LOADING DESENSITIZES VP GENE TRANSCRIPTION IN SON OF BORDERLINE HYPERTENSIVE RATS

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Introduction: Vasopressin (VP) is a key regulator of blood pressure and osmotic homeostasis. VP is primarily synthetized in magnocellular neurons (MCNs) of Paraventricular (PVN) and Supraoptic nucleus (SON) in hypothalamus. Animal models of genetic hypertension suggest significant change in VP hypothalamic levels. SON VP expression is shown to be particularly sensitive to high salt levels, since SON is more densely populated by plasma secreting MCNs, in comparison to PVN. While VP mRNA levels gradually change in response to acute osmotic stimulation, hnRNA levels are rapidly increased, indicating enhanced VP gene transcription.
Method: Wistar rats (WR) were used as normotensive controls, and Borderline hypertensive rats (BHR), genetically predisposed for developing hypertension, were randomized in two groups: first kept under baseline condition, and second salt loaded with 0.9 % NaCl for 25 weeks. All animals were age-matched. After decapitation, harvested brains were snap frozen at –80 °C. SON tissue was collected by punching method using a cryostat, and then prepared for PCR, to estimate the gene expression at hnRNA and mRNA levels, using a 2-ΔΔ Ct method. Correlation between hnRNA and mRNA expression was evaluated using Spearman's rho, while mRNA and hnRNA change in all groups was tested by Mann–Whitney test.
Results: Very strong positive correlation was observed between hnRNA and mRNA relative levels in baseline BHRs ($r = 0.89$, $p = 0.007$). When compared to WRs (median= 1.01, range = 0.87–1.38) VP mRNA expression showed increasing tendency without statistical significance, in BHRs under baseline conditions (median = 1.32, range = 0.55–4.40) and on salt loading (median = 1.99, range = 1.00–6.57). BHRs under baseline conditions (median = 2.56, range = 1.67–9.83) had significantly higher ($p = 0.008$) hnRNA expression compared to WRs (median = 1.09, range = 0.81–1.29). On contrary, chronic salt loading (median = 1.79, range = 0.85–2.47) decreased hnRNA expression ($p = 0.048$) in SON of BHRs, leveling it to values observed in WRs.
Conclusion: BHRs have basally higher VP gene transcription rate than WRs, due to their genetic constitution. The hnRNA synthesis positively correlates with mRNA levels in baseline BHRs. Chronic salt loading seems to blunt transcriptional sensitivity in BHRs, without disrupting the VP mRNA levels. This suggests chronic osmotic stimulation triggers post-translational mechanism that provide mRNA stability, optimizing the rate of VP translation and synthesis.

THE IMPACT OF THE DABIGATRAN AND NATTOKINASE ON THE REDOX STATUS IN ISOPROTERENOL-INDUCED HEART FAILURE

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Introduction: Heart failure (HF) represents a significant global health burden and remains a leading cause of hospitalization and mortality, particularly among older adults. A key contributor to the development and progression of HF is oxidative stress, which disrupts cardiac homeostasis by promoting myocardial dysfunction and pathological remodeling. In select HF populations, particularly those with concurrent atrial fibrillation, anticoagulant therapy is employed to reduce the risk of thromboembolism. These non-anticoagulant benefits remain under investigation and are not yet established in standard clinical practice. This study aimed to examine the effect of dabigatran (D) and nattokinase (NK) on redox status in isoproterenol-induced HF in rats.
Methodology: A total of 50 Wistar albino male rats were used in this study and randomly divided into five groups: CTRL – healthy rats; HF – rats with heart failure; NK – rats with HF treated with nattokinase (1000 Fu/kg bw per day); D – rats with HF treated with dabigatran (15 mg/kg daily); D+NK – rats with HF treated with combination of D and NK. HF was induced by subcutaneous administration of isoproterenol dissolved in saline at a dose of 5 mg/kg body weight per day for 7 consecutive days. D and NK were administered by gavage, every day, for 4 weeks. The parameters of redox status were evaluated from coronary venous effluent, blood samples and tissue samples.
Results: Our results demonstrated that D in combination with NK had the most prominent positive effect on redox status due to significantly improved prooxidative parameters and enhanced antioxidative capacity.
Conclusion: Given that HF has rising incidence, this research supplemented knowledge and given new insights about the effects of dabigatran and nattokinase on redox status in isoproterenol-induced HF.

MONOAMINE OXIDASE OVEREXPRESSION PERSISTS DESPITE GLYCEMIA NORMALIZATION AND CONTRIBUTES TO ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL DIABETES

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Introduction: Oxidative stress is a major driver of vascular complications in diabetes and monoamine oxidases (MAO-A and B) have emerged in the past decade as crucial contributors. These mitochondrial enzymes generate hydrogen peroxide during catecholamines metabolism, potentially exacerbating endothelial dysfunction. While insulin therapy normalizes blood glucose, its impact on diabetes-induced vascular changes, particularly MAO expression, remains unclear. The present study conducted in mice with diabetes mellitus (DM, type 1, induced with STZ, 50 mg/kg i.p.) was double aimed: i) to investigate if insulin therapy reverses MAO upregulation in aortic samples and ii) to assess the impact of MAO inhibition on vascular function and oxidative stress.
Methodology: Adult male mice were divided into 4 groups: Control (non-diabetic), Diabetic (DM, untreated for 4 weeks), Diabetic + Insulin (DM+INS, 2 weeks untreated + 2 weeks glargine, 10 U/kg/day), and Diabetic + Insulin + MAO inhibitors (DM+INS+MAOi, same insulin protocol + 1 week of clorgyline and selegiline, 1 mg/kg/day). Aortic MAO expression was quantified by qPCR and IHC. Oxidative stress was measured by confocal microscopy (DHE staining) and spectrophotometry (FOX assay). Vascular function was assessed by isometric tension recording using wire myography to evaluate endothelium-dependent relaxation (to ACh) and contractile responses (to Phe) in isolated aortic rings.
Results: MAO expression was significantly increased in diabetic aortic samples after 2 weeks of hyperglycemia and remained elevated despite 2 weeks of insulin-induced glycemic normalization. Myograph studies revealed impairment of the endothelium-dependent relaxation in DM and DM+INS groups despite normalized blood glucose in the latter. Importantly, co-administration of MAOi (in DM+INS+MAOi group) significantly improved endothelium-dependent vasodilation and reduced vascular oxidative stress. These improvements occurred without further changes in glycemia, indicating a direct vascular benefit of MAO inhibition.
Conclusion: In conclusion, MAO expression remains elevated in diabetic vessels despite insulin-mediated glycemic control and contributes to persistent endothelial dysfunction and oxidative stress. Short-term MAO inhibition restores vascular function, supporting its potential role as an adjunct therapy to insulin for mitigating diabetic vasculopathy.

CONCLUSIONS FROM 20 YEARS OF CELLULAR AP RECORDINGS: AP PARAMETER CORRELATIONS, SOLUTION-, AGE-, AND SEX-DEPENDENCE

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Dog is a good model of human due to similar action potential (AP) shape, transmural difference and rate-dependence. Sex-difference is an important issue to be addressed in research when analyzing results. We compared AP parameters recorded at steady-state pacing with sharp microelectrodes using different solutions (Krebs, BTY and NTY) and evaluated the sex- and age-dependence of the APs of canine left ventricular myocytes.
Experiments were done on enzymatically isolated left ventricular myocytes obtained from young adult dogs at 37°C. AP parameters were determined in 10 consecutive APs and then averaged. Determination of transmural origin of the cells (Epi, Mid and Endo) was based on their AP shape especially on phase 1, its maximal rate, and presence of spike and dome configuration.
AP duration (APD) of Epi cells was always smaller than that of Mid cells. There was no transmural difference in resting membrane potential (RMP) values. Amplitude of the AP (APA) and its early plateau of Endo cells was higher than in Mid or Epi cells, respectively. Transmural differences of AP parameters were more similar in BTY and NTY solutions than in Krebs. The lowest values of RMP and plateau potentials were seen in Krebs for Epi, Mid and Endo cells too. Moreover, in Mid cells APD, APA and peak values were also lower in Krebs than in the other two solutions. No difference was seen between cells obtained from males and female animals in Phase1 amplitude and maximal rate of final repolarization (V max). In Mid cells maximal rate of early repolarization (VPh1max), early plateau potential, APA and peak values were also the same in both sexes. APD values of late repolarization were larger whereas late plateau potentials were smaller in Mid cells of males. In male Epi cells maximal rate of depolarization was smaller than in females. Weak correlation was detected between age and APA or peak values but only in Epi cells. APD values at different repolarization values strongly correlated with each other, just as early and mid-plateau potentials. Strong negative correlation was detected between V_{max} and APD50/APD90 values. Peak and plateau 50 AMP values showed binomial distribution only in some perfusion solutions and in certain cells.

To conclude, AP parameters shown hardly any age-dependence but slight differences in various perfusion solutions. Endo cells are more similar to Mid than Epi cells. Strong correlations were found among AP parameters characteristic for similar AP phases.

LEVOSIMENDAN PRETREATMENT PRESERVES INTESTINAL TISSUE DAMAGE AND MULTI-ORGAN INJURY BY ATTENUATING OXIDATIVE STRESS, INFLAMMATION AND APOPTOSIS INDUCED BY MESENTERIC ARTERY ISCHEMIA AND REPERFUSION

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Background: Acute mesenteric ischemia (AMI) is characterized by oxidative stress, inflammation, apoptosis and necrosis of intestinal epithelial cells associated with multi-organ injuries. Levosimendan is a drug with proven anti-ischemic effects used in the management of acutely decompensated congestive heart failure. This study evaluated the protective effects of levosimendan pretreatment on intestinal tissue and multi-organ damage in a rat model of mesenteric artery ischemia and reperfusion (I/R).
Methodology: The rats were divided into four groups. The sham group was a control, the I/R group was used for AMI, the levosimendan group received levosimendan (1 mg/kg. i.p.) and the levosimendan + I/R group received levosimendan (1 mg/kg, i.p.) 30 minutes before the onset of I/R injury. Oxidative stress markers were assessed in plasma, erythrocyte lysate, broncho-alveolar lavage fluid (BALF) and in ileum tissue homogenate. Histopathological examination and morphometric analysis were assessed in the tissue samples of small intestines, lungs and heart. Immunohistochemical staining was used to identify the presence of immune cell infiltrate, inflammatory and oxidative stress-related mediators, as well as the markers of apoptosis.
Results: The I/R by itself caused elevation of oxidative stress markers, induced tissue inflammation, and apoptosis. Levosimendan pretreatment significantly reduced these markers and enhanced antioxidant defense system (SOD, CAT, GSH) in intestinal tissue and in BALF. Histological analysis revealed reduced mucosal damage, as well as lung cells and cardiomycytes damage. Immunohistochemical staining showed that levosimendan pretreatment significantly reduced apoptosis (TUNEL, CC3) of intestinal epithelial cells, decreased markers of inflammation (NF-kB, IL-6, CD68) and induced upregulation of anti-inflammatory proteins like Nrf2 and HO-1.
Conclusion: These findings highlight levosimendan's ability to protect intestinal mucosa and multi-organ damage during I/R by activating the Nrf2/HO-1 pathway and suppressing proinflammatory and proapoptotic signaling.

THE IMPORTANCE OF SEX DIFFERENCES IN LEFT VENTRICULAR REMODELING FOR RISK STRATIFICATION OF PATIENTS WITH AORTIC REGURGITATION

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Background: Left ventricular (LV) dilatation is an established prognosticator in aortic regurgitation (AR). Current guidelines recommend using LV end-systolic diameter index (LVESDi) to assess the need for aortic valve surgery (AVS), applying the same threshold of 25 mm/m^2 regardless of sex. However, LV volumes have been suggested to better depict LV remodeling in AR.
Purpose: To assess sex differences in LV remodeling using linear and volumetric measurements in patients with moderate-severe AR and their association with outcomes.
Methods:1070 patients (56 ± 18 years, 691 men) with moderate-severe AR were included. The primary outcome was all-cause mortality.
Results: Women were older (58 ± 19 vs. 55 ± 17, $p = 0.023$) and had more advanced HF symptoms than men (NYHA class III–IV 12% vs. 9%, $p = 0.017$). Men showed larger LVESDi (21 ± 5 vs. 20 ± 5 mm/m^2, $p = 0.013$) and LV end-systolic volume index (LVESVi 37 ± 28 vs. 26 ± 17 ml/m^2, $p < 0.001$).
During a median follow-up of 89 (IQR 54–132) months,168 patients died. Women had lower survival rates at 10 years

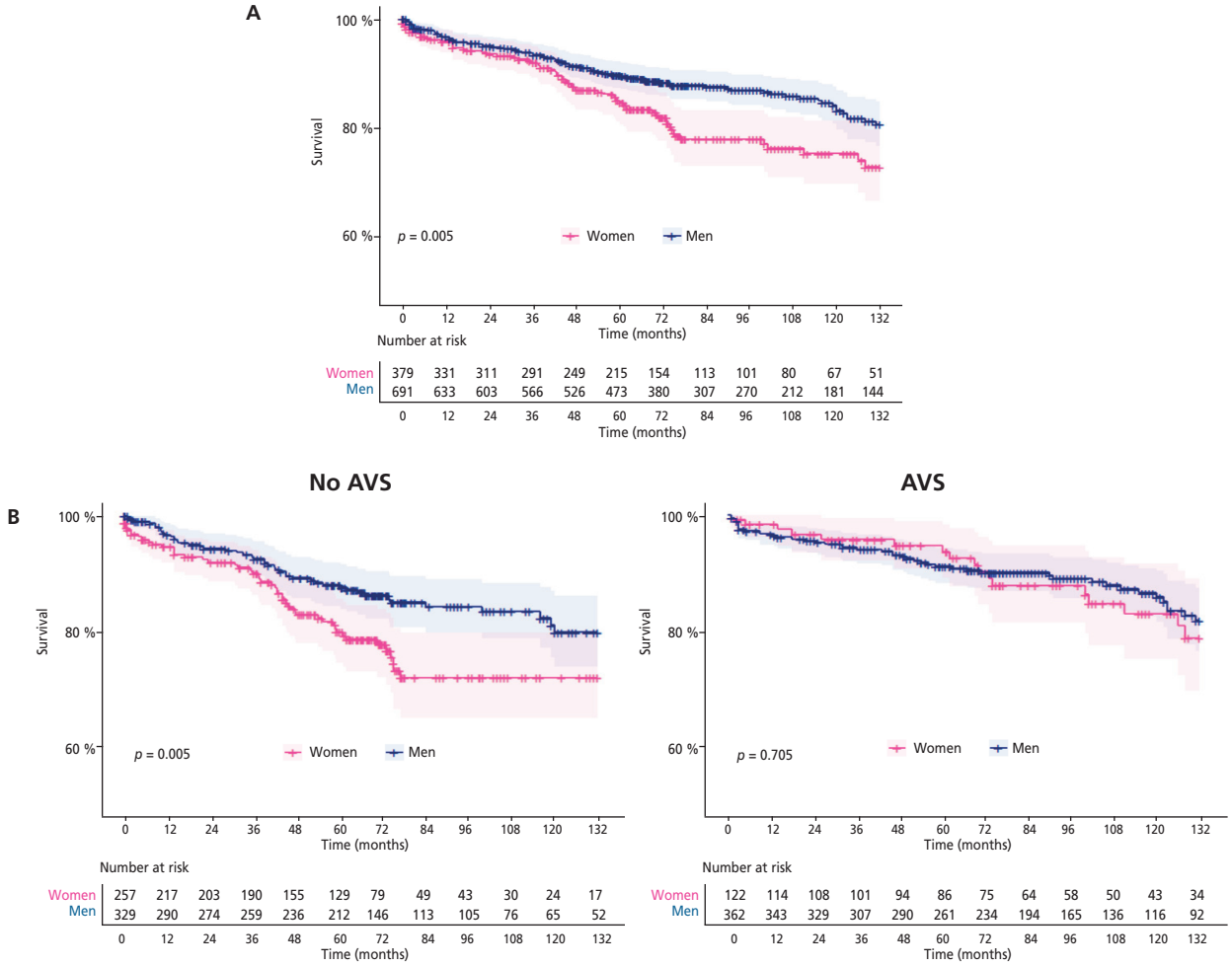


Fig. 1 (A) Kaplan–Meier curves for all-cause mortality according to sex; (B) Kaplan–Meier curves for all-cause mortality according to sex and AVS.

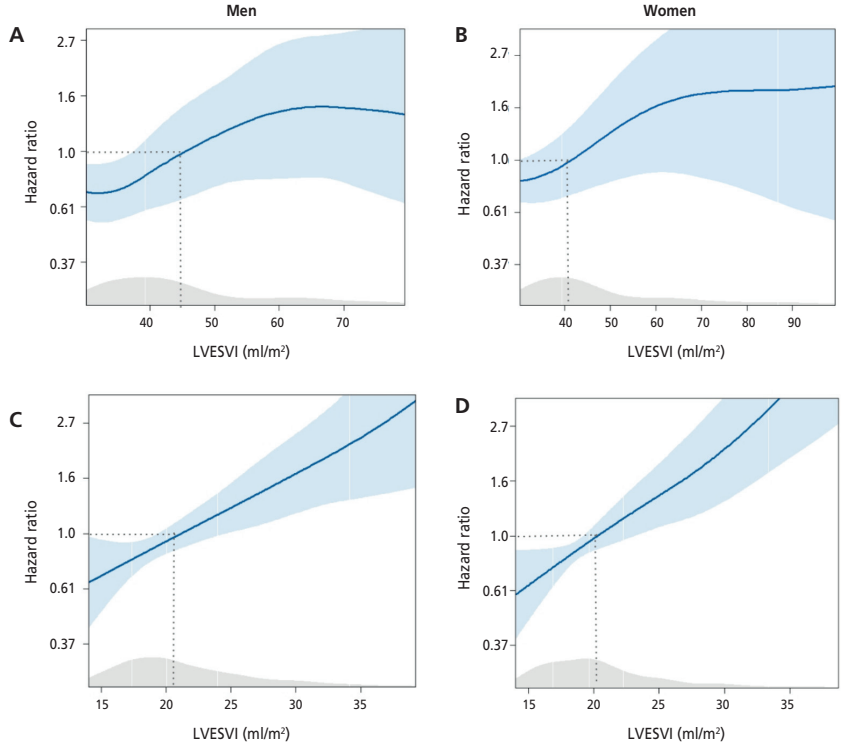


Fig. 2 – Penalized spline curves for all-cause mortality according to LVESVi and LVESDi values. The spline curve illustrates the HR change for the primary endpoint with 95% CIs (shaded blue areas). (A) LVESVi spline curve for men adjusted for age; (B) LVESVi spline curve for women adjusted for age; (C) LVESDi spline curve for men adjusted for age; (D) LVESDi spline curve for women adjusted for age.
LVESVi – left ventricular end-systolic volume index.

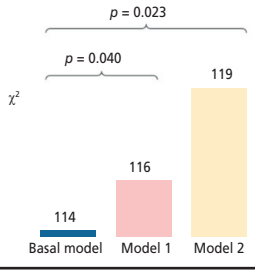
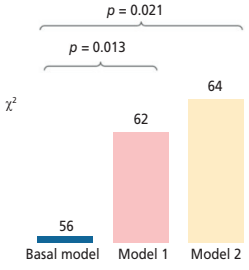
	A. Multivariate Cox regression				B. Likelihood ratio
	Model 1: LVESDi HR (95% CI)	*p*-value	Model 2: LVESVi HR (95% CI)	*p*-value	
Women					
Age	1.043 (1.024–1.063)	**<0.001**	1.045 (1.025–1.065)	**<0.001**	
NYHA class III–IV	2.137 (1.228–3.720)	**0.007**	2.017 (1.124–3.618)	**0.019**	
AVS*	0.483 (0.255–0.913)	**0.025**	0.422 (0.212–0.839)	**0.014**	
LVEF <55%	1.695 (1.034–2.785)	**0.007**	1.809 (1.065–3.071)	**0.028**	
LVESDi >20 mm/m²	1.710 (1.003–2.916)	**0.006**			
LVESVi >40 ml/m²			1.968 (1.119–3.463)	**0.019**	
Men					
Age	1.064 (1.045–1.084)	**<0.001**	1.066 (1.047–1.086)	**<0.001**	
NYHA class III–IV	1.876 (1.117–3.152)	**0.017**	1.767 (1.048–2.979)	**0.033**	
AVS*	0.391 (0.238–0.642)	**<0.001**	0.373 (0.226–0.616)	**<0.001**	
LVEF <55%	2.041 (1.232–3.379)	**<0.001**	1.871 (1.097–3.191)	**0.021**	
LAVI ml/m² (per 5 ml increase)	1.029 (1.001–1.058)	**0.042**	1.029 (1.001–1.058)	**0.044**	
LVESDi >20 mm/m²	1.638 (1.015–2.643)	**0.043**			
LVESVi >40 ml/m²			1.775 (1.076–2.928)	**0.025**	

Table 1 – Multivariate Cox regression analysis for all-cause mortality for men and women (A). Likelihood ratio: the presence of volumetric LV dilatation (Model 2) demonstrates a higher incremental prognostic value compared to LVESDi >20 mm/m² (Model 1) when added to a basal model for women, which included age, NYHA class III–IV, LVEF <55%, and AVS*, and basal model for men, which included age, LVEF <55%, AVS*, and LAVI (B).

compared to men (75.3% vs. 84.1%, $p = 0.005$) (**Fig. 1A**). However, sex mortality difference was no longer significant when patients were treated surgically ($p = 0.705$) (**Fig. 1B**).Spline curve analysis revealed that the LVESDi threshold associated with an increased risk of mortality was 20 mm/m2 for both sexes (**Fig. 2**). The LVESVi threshold was 40 ml/m2 for women and 45 ml/m2 for men (**Fig. 2**). LV dilatation defined by these cut-offs remained independently associated with mortality after adjusting for clinical and echocardiographic variables (**Table 1A**).

Conclusion: In moderate-severe AR, similar LVSEDi thresholds (20 mm/m$^{2)}$ for both sexes, but lower than currently recommended by guidelines, were associated with mortality. For LVESVi, lower thresholds for women compared to men (40 ml/m² vs. 45 ml/m²) were associated with worse survival.

ROLE OF LYSOPHOSPHATIDYLCHOLINE IN ISCHEMIA-REPERFUSION INJURY

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Introduction: It is now well established that bioactive phospholipid metabolites including lysophosphatidylcholine (LPC) accumulate in cardiac tissue under conditions of ischemia-reperfusion. Due to its amphiphilic property and interaction with the cell membrane; LPC may alter SL membrane associated Ca^{2+}-handling proteins and thus contribute to the occurrence of Ca^{2+}-overload and subsequent cardiac injury and functional abnormalities.

Methods: We have explored the mechanisms whereby LPC-induces an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) in isolated adult rat cardiomyocytes.

Results: LPC was observed to increase $[Ca^{2+}]i$ in a dose-dependent manner. The LPC -induced increase in $[Ca^{2+}]$ i was augmented with increasing the concentration of extracellular Ca^{2+} and was abolished by the removal of Ca^{2+} from the medium. Pre-treatment of cardiomyocytes with verapamil did not affect the LPC-evoked increase in $[Ca^{2+}]i$. However, inhibition of $Na^{+-}K^{+}$ ATPase with ouabain potentiated the LPC response, while the LPC-induced increase in $[Ca^{2+}]i$ was attenuated by $Na^{+-}Ca^{2+}$ exchanger inhibitors. Depletion of SR Ca^{2+} stores by ryanodine or by thapsigargin depressed the LPC-mediated increase in $[Ca^{2+}]i$. Blockade of the Na+-Ca2+ exchanger and inhibition of SR Ca^{2+}-pump or ryanodine receptor had an additive inhibitory effect on the LPC-induced increase in $[Ca^{2+}]i$.

Conclusion: These observations suggest that the increase in $[Ca^{2+}]i$ induced by LPC depends on both Ca^{2+}-influx from the extracellular space and Ca^{2+}-release from the SR stores. Therefore, prevention of LPC action or its accumulation could represent a new approach for cardioprotection against ischemia/reperfusion damage.

IMPAIRED AUTOPHAGY MEDIATES DOXORUBICIN CARDIOMYOPATHY THROUGH THE CGAS-STING INFLAMMASOME PATHWAY

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Doxorubicin (Dox) is widely used to treat a wide variety of human malignancies, however, a major side effect is its propensity for inducing heart failure. The mechanisms by which Dox triggers cardiac dysfunction are poorly understood. Previous studies have revealed that cellular quality control processes such as autophagy play a critical homeostatic role by clearing oxidized proteins and damaged organelles such as mitochondria through a lysosomal pathway. In this report, we investigated whether the reported cardiotoxic effects of Dox were related to impaired autophagy in cancer patients with ejection fraction of < 30% that had been treated with Dox. While investigating the underlying mechanisms for increased damaged mitochondria in Dox-treated hearts, we assessed the autophagy markers and observed increased expression levels of key autophagic proteins such as LC3II, Beclin-1, and ATGs. However, the increased p62 levels in the Dox-treated hearts, indicate the lack of autophagic clearance. The impaired autophagy resulted in the accumulation of undigested autophagosomes and cellular debris that triggered the activation of the Damage Activation Molecular Program (DAMP) response resulting in the cGAS-STING pathway. This coincided with the formation of NRLP3 inflammasome activation and widespread cardiac cell death. Notably, interventions that inhibited STING activation suppressed NRLP3 inflammasomes, mitochondrial injury, and cell death following Dox treatment. Notably, autophagy inhibitors 3-Methyl adenine which block the class III PI3K-mTOR pathway or Atg7 knock-down suppressed cell death induced by Dox. Hence, the findings of the present study demonstrate that the accumulation of undigested autophagosomes in cancer patients treated with Dox serve as DAMPs to trigger the activation of cGAS-STING and NRLP3 inflammasomes which subsequently result in cardiac cell death. Our data suggest that therapeutic interventions that modulate autophagy flux may prove beneficial to preserving cardiac function and mitigating cardiotoxicity in cancer patients treated with Dox.

SUBSTANCE XXX – AN UNKNOWN COMPOUND SIMILAR TO N, N-DIMETHYLTRYPTAMINE IN PIG SERUM AFTER EXPERIMENTAL CARDIAC ARREST

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Aim: The aim of our research was to determine whether N,N-dimethyltryptamine (DMT) is present in the blood of pigs after experimentally induced cardiac arrest. Our hypothesis is that this psychedelic compound could be responsible for near-death experiences (NDE) in humans due to the similarity of effects. Additionally, this substance might have a protective role not only on the psyche but also on brain tissue via sigma receptors. We began our study using pigs in which the effect of ECMO (extracorporeal membrane oxygenation) was being examined.
Method: In two pigs connected to ECMO we collected a series of blood samples from the central vein before, in and after experimental cardiac arrest – a total of 20 samples at five-minutes intervals. Ventricular fibrillation was induced in the 15th minute and ECMO was initiated after an additional 15 minutes to restore circulation. Samples were immediately centrifuged and the serum was frozen at –80 grades of Celsia.
We searched the serum for the folowing substances: tryptophan, serotonin, tryptamine, N,N-dimethyltryptamine (DMT), 5-methoxy-DMT (5-meo-DMT), DMT-NO, 5-OH-DMT, psilocin and 3-hydroxyindolacetic acid (3-IAA).
Results: Measurable levels of tryptophan, tryptamine, serotonin, and 3-IAA were found in the serum of pigs after cardiac arrest. At the moment of induced cardiac arrest, a low concentration of an unknown compound appeared, exhibiting some charakteristic similar to DMT. This compound (XXX) is capable of fragmenting at m/z 58, has retention time one minute shorter than DMT, and is more polar than DMT. We performed statistical processing-time curves of measurable substances, ratio analyses of IAA/serotonin, IAA/tryptophan, IAA/XXX, serotonin/tryptophan and serotonin/tryptamine. We made the correlation analyses also.
Conclusion: The detection of a DMT-like substance XXX at the moment of cardiac arrest raises several hypotheses. There is no N,N-dimethyltryptamine and other known substances with psychedelic effect. Identifying compound XXX could lead to new insights into pathophysiology and potentially adjust protocols to better protect brain tissue following cardiac arrest. Demonstrating the presence of this compound in human serum after cardiac arrest will be necessary for further validation.

NEW ADVANCES IN COMPUTATIONAL MODELLING OF THE HEART

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Introduction: Computational modelling of cardiac physiology is an increasingly integral technique in cardiovascular

research and pharmaceutical industry. Human-specific virtual cardiomyocytes avoid the limitations of animal models resulting from species differences, as well as the currently immature phenotype of hIPSC-CM and organoids. How can we use computer modelling to advance our understanding of cardiac disease, or to predict safety and efficacy of candidate drugs?
Methods: I will illustrate the utility of computational modelling in cardiology using the first highly general human virtual cardiomyocyte T-World, which we recently developed.
Results: T-World quantitatively reproduces human ventricular physiology, from electrical activation to contraction, and is the first to replicate all key cellular mechanisms driving life-threatening arrhythmias. Extensively validated on unseen data, it demonstrates strong predictivity across applications and scales. It can be used to predict safety of drugs, and to investigate mechanisms of electrical or mechanical dysfunction in complex diseases, such as Type 2 diabetes or both types of heart failure.
Conclusions: T-World is a versatile and validated tool applicable from basic science to industry. Upon publication, the model will be freely available, including through an online graphical user interface.

CARDIAC ELECTROPHYSIOLOGICAL EFFECTS OF CHRONIC TESTOSTERONE ADMINISTRATION: INSIGHTS FROM CANINE *IN VIVO* AND *IN VITRO* STUDIES

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Background: Chronic use of androgenic anabolic steroids is thought to cause electrophysiological cardiac remodeling; however, the cardiac effect of supra-physiological testosterone level is not fully understood yet. Therefore, our study aimed to investigate the potential electrophysiological effects of chronic testosterone-undecanoate administration in a canine model.
Methods: Male Beagle dogs were randomized into control ('Cont') and treated ('Tr') groups (n = 9 per group). The latter group received 15 mg/kg of long-acting testosterone-undecanoate intramuscular injections weekly for 3 months. To investigate the cardiac electrophysiological effects, in vivo and in vitro studies were performed.
Results: The testosterone level was significantly higher in the 'Tr' group compared to the 'Cont' group (47.02 nmol/L vs. 15.23 nmol/L). The chronic treatment resulted in significantly shortened QT (226 ± 49 vs. 244.9 ± 26.6 ms; p <0.05) and QTc (26.06 ± 2.5 vs. 29.6 ± 3.01 ms, p <0.05) intervals. The imprint of the in vivo observed electrophysiological changes were also presented at the tissue and cellular levels, since the APD90 of left ventricular myocytes (235.2 ± 26.7 vs. 283.6 ± 28.5 ms; p <0.05) and mid-myocardial slice were significantly shortened in the 'Tr' group. Patch-clamp experiments revealed an increased magnitude of Ito, IK1, and IKs in the 'Tr' group. Besides that, the ECG recordings also presented prolonged PQ (112.1 ± 15.5 vs. 61.65 ± 12.7ms; p <0.05) and QRS intervals (72.37 ± 15.4 vs. 61.65 ± 12.7 ms; p <0.05) in the 'Tr' group.
Conclusion: The cardiac electrical remodeling induced by a constant high level of testosterone seemingly shares similarities with short QT-syndromes, partly due to the greater amplitude of the investigated potassium currents. These changes may contribute to the development of life-threatening arrhythmias under certain circumstances.

PRECISION IN PRACTICE: DIRECT BIOCHEMICAL MONITORING OF DRUG EFFICACY FOR PERSONALIZED CARDIOVASCULAR THERAPY

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Background: Despite advances in pharmacotherapy, fewer than 50% of cardiovascular patients adhere effectively to prescribed medications – undermining efforts to reduce morbidity and mortality. A major gap in current clinical practice is the absence of tools to confirm whether a given drug dose is achieving its intended biological effect.
Objective: We aimed to develop and clinically validate biochemical assays capable of directly measuring the pharmacodynamic efficacy of commonly prescribed cardiovascular medications, with the goal of optimizing individualized treatment strategies.
Methods: Novel, target-specific assays were developed to quantify the biochemical effects of three major drug classes: angiotensin-converting enzyme (ACE) inhibitors, direct oral anticoagulants (DOACs), and dipeptidyl peptidase-4 (DPP4) inhibitors. These assays were applied in a cohort of cardiovascular patients undergoing routine care.
Results: Measured ACE inhibition showed a significant correlation with blood pressure response, supporting its potential for dose optimization. Factor Xa activity, reflect-

ing DOAC efficacy, emerged as the most sensitive and informative biochemical marker to date – revealing cases of both subtherapeutic exposure and potential overdosing. The observed great variability in drug responses suggests a need for closer therapeutic monitoring in cardiovascular patients.
Conclusion: Direct biochemical assessment of drug efficacy enables a new paradigm in personalized medicine – transforming prescription into precision. Rather than relying solely on dose and timing, clinicians can now guide therapy based on verified biological effect. This approach empowers both patients and care teams to reach therapeutic targets between visits, improving outcomes while minimizing risks.

CONNEXIN 43 AS A NOVEL CALCIUM TRANSPORT PATHWAY IN THE NUCLEAR ENVELOPE OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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The plasticity of cardiac calcium signaling emanates from interplay of the SR, mitochondrial and nuclear envelope calcium stores. Cardiac contraction is mediated primarily by Ica triggered Ca2+ release from the SR ryanodine receptors (RyR2s), a mechanism called calcium-induced calcium-release (CICR). The contribution of mitochondria and nucleus to EC-coupling or excitation-transcription coupling, however, remains not fully understood. Functional evidence for storage and release of nuclear calcium comes from Mag-fluo-4 (Kd = 20 μM) loaded cardiomyocytes, where 20 mM caffeine reversibly abolished nuclear Mag-fluo-4 fluorescence signals in absence of RyR expression in the nucleus. Other studies report presence of IP3Rs in the nuclear envelop membranes that could induce IP3 mediated Ca2+ release, suggesting that nuclear calcium maybe regulated independent of the cytosolic calcium. Here we investigated the nuclear envelop-associated Ca2+ signals using a genetically engineered fluorescent Ca2+ probe targeted to nuclear Lamin-B at the inner nuclear envelop (NE) membrane. Confocal imaging showed rapid and larger (2-5X) Ca2+ rise in the nuclear lamina using GCaMP6s targeted to Lamin-B in response to Ica-triggered SR Ca2+ release. Application of caffeine induced even larger (2-10X) Ca2+ rises in Lamine-B signals. RyR2 mutations (E3848A and Q3925E), that disabled Ica- and caffeine-triggered SR Ca2+ release greatly suppressed NE Ca2+ signals, suggesting SR Ca2+ release is required for NE Ca2+-signaling. In CICR-disabled mutants where caffeine failed to activate cytosolic Ca-transients, robust spontaneously activating NE Ca2+ transients persisted, suggesting involvement of alternate Ca2+-signaling pathway that compensates for CICR loss. We identified robust expression of Cx43 in the NE, the inhibition of which by Gap19 protein blocked the rise of nuclear lamina Ca2+ transients. Conclusion: while SR Ca2+ release is essential in replenishing and activating NE Ca2+ signals, mutations in RyR2 that disable CICR remodel calcium signaling pathway such that other pathways that include endogenously expressed Cx43 in NE to maintain the calcium fluxes critical for spontaneously activated Ca-transients and pacing.

EXAMINATION OF CARDIAC EFFECTS OF MODERN ANTIDIABETIC TREATMENT ON PHYSICAL INACTIVITY AND OBESITY--INDUCED HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFPEF) ANIMAL MODEL

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Introduction: In economically developed countries, the incidence of heart failure is steadily increasing, with approximately 3 new cases per 1,000 people each year. About half of these cases are classified as heart failure with preserved ejection fraction (HFpEF). However, our understanding of the exact pathomechanism underlying HFpEF remains limited. Recent clinical trials, such as DELIVER and SELECT, have demonstrated that certain newer antidiabetic agents, including SGLT-2 inhibitors and GLP-1 receptor agonists, can significantly reduce cardiovascular complications, even in individuals without diabetes. Although the precise mechanisms by which these drugs confer cardiovascular protection are not yet fully understood, their anti-inflammatory and antioxidant effects – possibly mediated by mitochondrial modulation – appear to play a key role.
Methodology: In our experiment, we aimed to develop a multi-hit model in which CD-1 mice were exposed to etiological factors analogous to those in human pathology. The animals were maintained on a high-fat diet (22% fat) and their available movement space was restricted to simulate sedentary conditions. One group received empagliflozin and another semaglutide throughout the study. After 3 months, the animals underwent echocardiography and NT-proBNP measurement.
Results: The results clearly demonstrate that in the HFpEF group, myocardial wall thickness increased significantly (posterior wall: mm: 0,82 vs 1,042; septum: mm: 0,76 vs 1,103), while no significant changes were observed in control or treated groups. Several diastolic parameters also deteriorated: e' velocity decreased (ms: 25,89 vs 20,3), deceleration time shortened (31,9 ms vs 21,9 ms), E/A ratio

declined (2,71 vs 2,12), and E/e' increased (25,5 vs 32,4). These changes were absent in the control or treated animals. No significant differences were found in ejection fraction or left ventricular cavity diameter. NT-proBNP levels were also significantly elevated in the HFpEF group (pg/ml: 141,7 vs 76,51, 68,65, 66,83).
Conclusion: The observed diastolic dysfunction – evidenced by echocardiography and elevated NT-proBNP, without changes in ejection fraction – confirms the HFpEF phenotype after 3 months. Both SGLT-2 inhibitors and GLP-1 receptor agonists effectively attenuated these alterations. This protective action is likely mediated by complex mechanisms, which we aim to explore in future studies, particularly regarding mitochondrial function.

CARDIOVASCULAR ONCOLOGY: CANCER--THERAPY RELATED HYPERTENSION

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Modern chemotherapies have improved cancer survival at the expense of cardiovascular toxicities. One of the most common side effects of these drugs is hypertension, especially in patients treated with vascular endothelial growth factor (VEGF) inhibitors. Exact molecular mechanisms underlying hypertension are unclear, but recent discoveries indicate an important role for oxidative stress, ET-1, prostaglandins, endothelial dysfunction, increased sympathetic outflow, and microvascular rarefaction. This presentation will provide new insights into anti-cancer drug-induced hypertension and will focus on some underlying mechanisms and therapeutic implications.

CARDIOVASCULAR EFFECTS OF FOUR--WEEK CURCUMIN TREATMENT ON THE HEART OF WISTAR RATS IN A MODEL OF EXPERIMENTAL RHEUMATOID ARTHRITIS

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Introduction: Curcumin, strong active principle from turmeric, has potential therapeutic effect in treatment of many diseases due to its anti-inflammatory and antioxidant activity. In rats, both curcumin pretreatment and post-treatment can offer protective effects, but their mechanisms and efficacy can differ depending on the specific context and condition being studied. The aim of this study was to compare pretreatment and post-treatment of curcumin in treatment of RA and its antioxidant effects.
Materials and methods: Adjuvant-induced arthritis model was developed by using 0.1 ml of Complete Freund's Adjuvant (CFA) subcutaneously at the base of the rat tail. In this study, 18 Wistar albino rats were divided into five groups: healthy rats (control group), rats with CFA-induced RA (RA group) and rats with CFA-induced RA treated with curcumin in dose 200 mg/kg three times a week for 4 weeks per os (RA+CUR group), rats pretreated with curcumin before CFA induction for four weeks (PrCUR+RA) without and with MTX treatment (PrCUR+RA+MTX). Coronary function was estimated according to the retrograde Langendorff perfusion by measuring the parameters of cardiodynamics and coronary circulation.
Results: Curcumin reversed the dynamics of parameters of contractility in RA+CUR group compared to the RA group. Pretreatment with curcumin improved heart function in rats with RA stronger that in PrCUR+RA group.
Conclusion: Curcumin's ability to reduce inflammation, promote antioxidant defenses in pretreatments, and enhancing the tissue repair thus contribute to its cardioprotective effects in post-treatment scenarios.

OFF-TARGET EFFECTS OF A DUAL GLP-1/ GIP RECEPTOR AGONIST VIA MODULATION OF THE β-ADRENOCEPTORS AND GLUCOSE METABOLISM IN CARDIAC CELLS

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Tirzepatide (TZPD), a GIP and GLP1 receptor agonist, is a novel cardioprotective agent, particularly in metabolic disturbances-related co-morbidities, however, there is no exact study to emphasize its possible off-target action in cardiac cells. Considering a trafficking of incretin receptors in a manner not anticipated by the standard way of cAMP as a primary actor in TZPD action, together with the role of cAMP depression in cardiac dysfunction, here, we aimed to elucidate a pattern of off-target receptor interactions of TZPD and molecular processes underlying the pleiotropic effects of TZPD through modulation of the β-adrenoceptors (β-ARs) signaling in cardiomyocytes. To establish the multifaceted cardioprotective function and underlying mechanisms of TZPD against hyperglycemia (HG)- or senescence (SC)-induced cardiac dysfunction, H9c2 cells were treated with and without TZPD. We also used β_3-AR-overexpressed H9c2 cells (β_3OE) for comparison. The TZPD intervention ameliorated the HG or SC phenotypes in the cardiac cells via

alleviation in protein levels of GLP-1R and GIP-R as well as production of cAMP or cGMP even in the presence of these receptor antagonisms. The TZPD also alleviated the depressed levels of the β_1- and β_2-ARs, accompanied by a significant decrease in activated β3-ARs and PKG, which paralleled normalizations in cAMP and cGMP in the presence of receptor antagonists. The therapeutic effects of TZPD on similar parameters of the β_3OE group of cells can strongly verify its off-target action among multifaceted effects in either HG or SC cells. In addition, molecular dynamics simulations indicated that TZPD binds with the highest affinity to GLP-1R and β_3-ARs rather than GIP-R and then relatively lower affinities to β_1- and β_2-ARs. Furthermore, mechanistically, the cardioprotective effect of TZPD includes significant regulation of the cellular Ca^{2+}, at most, modulating the proteins in β-ARs signaling pathways. Moreover, TZPD could significantly increase not only the depressed protein level but also the translocation of GLUT4 on the sarcolemma, promoting glucose uptake in HG or SC groups, independent of its receptor action. Our findings indicate that TZPD has beneficial effects by positively modulating β-AR signaling and glucose metabolism, rather than through on-target receptor action. Furthermore, we demonstrated how TZPD can engage the different targets with distinct signaling motifs at the sarcolemma.

CORRELATION OF HEART FAILURE BIOMARKERS AND MARKERS OF VASCULAR REMODELING WITH PROGNOSTIC SCORES IN OUTPATIENTS WITH CHRONIC HEART FAILURE

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Background: Heart failure biomarkers are useful tool in the estimation of outcome in chronic heart failure. The role of biomarkers of vascular remodeling in patient's outcome prediction has not yet been widely studied.
Study objective: To assess correlation of heart biomarkers together with markers of vascular remodeling with prognostic scores.
Patients and methods: The concentration of heart biomarkers: NT-proBNP, copeptin, cystatin-C, MR-proADM (midregional pro-adrenomedulin) and biomarkers of vascular remodeling apelin, ADMA (asymmetric dimethyl-arginin), and sMCAM/sCD146 (soluble melanoma cell adhesion molecule) was measured in outpatients together with examination of clinical, echocardiographic and other laboratory parameters. The patient's prognosis was estimated by Maggic (Meta-Analysis Global Group in Chronic Heart Failure and Get With the Guidelines-Heart Failure (GWTG-HF) scores. The patients were followed 18 months and the primary endpoints was hospitalization or death/transplant//LVAD.
Results: Total consecutive 79 patients from tertiary care HF clinic mean age 66 years, NYHA III-IV, mean LVEF 28 % and mean NT-proBNP 2276 pg/ml were enrolled in the study. The mean MAGGIC score was 21.7 points and mean GWTG-HF score was 37.7 points. After 18 months of follow-up, 27 patients (34%) were admitted to hospital and 15 patients (19 %) met the endpoint death/Tx/LVAD. Concentration of NT-proBNP, copeptin and cystatin C correlated with MAGGIC and GWTG-HF scores (p <0.001 for both) and concentration of apelin and sMCAM/sCD146 correlated with MAGGIC score (p <0,001 and p = 0,003) respectively. The levels of NT-proBNP, copeptin, cystatin C, sMCAM/sCD146 and ADMA were significantly higher in subjects with endpoint hospitalization or death/Tx/LVAD than in patients without endpoint.
Conclusion: Heart failure biomarkers NT-proBNP, copeptin and cystatin C and biomarkers of vascular remodeling apelin and sMCAM/sCD146 are associated with outcome of outpatients with chronic heart failure.

EFFECT OF A SELECTIVE IKUR INHIBITOR XEN-D0103 ON DOG AND HUMAN CARDIAC VENTRICULAR AND PURKINJE FIBER

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Introduction: Xention/Servier pharmaceutical companies developed XEN-D0103 as a selective inhibitor of IKur/Kv1.5 current to treat atrial fibrillation. The design of XEN-D0103 was motivated by the general consensus that IKur is expressed and functional primarily in atrial tissue, with minimal presence in ventricular tissue. Consequently, it was anticipated that XEN-D0103 would circumvent the proarrhythmic side effects commonly linked to the currently available therapies. However, a previous study casted doubt on whether IKur is actually absent from the ventricles. Therfore we studied the possible existence and function of IKur within the cardiac ventricular muscle.
Methods: Immunocytochemistry was utilized to detect the potential expression of Kv1.5 channels in isolated canine ventricular myocytes. Action potentials were recorded using standard conventional microelectrode techniques from both canine right ventricular papillary muscle and Purkinje fibers. Patch-clamp measurements were conducted to explore the impact of XEN-D0103 on isolated canine ventricular myocytes.
Results: Immunocitochemical analysis revealed abundant expression of Kv1.5 channels in canine ventricular

myocytes. Action potential recordings demonstrated a significant prolongation of APD90 in both canine ventricular 280.7 (± 7.5 ms vs. 292.0 ± 7.0 ms, n = 10, p ≤0.05) and Purkinje fibers (230 ± 9 ms vs. 258 ± 9 ms, n = 8, *p* ≤0.005) after treatment with 1 µmol XEN-D0103. Patch-clamp experiments showed that 1 µmol XEN-D0103 inhibits IKur in cardiac ventricular and Purkinje myocytes but does not inhibit IKr, IKs, Ito, or IK1 currents. However, at higher concentrations (3, 10 and 30 µmol), XEN-D0103 showed some inhibitory effects on Ito, IKr, IKs, and IK1 currents.
Conclusion: Contrary to the prevailing belief, our experimental findings suggest that IKur is indeed expressed within ventricular muscle and its inhibition can lenghten cardiac ventricular repolarization.

OXIDATIVE STRESS AND IMMUNE/INFLAMMATION IN METABOLIC SYNDROME: THE EFFECT OF ROSUVASTATIN

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Background: Oxidative stress plays a pivotal role in the pathogenesis of cardiovascular disease (CVD), most of which share metabolic risk factors. Among many medications, the impact of statins – particularly rosuvastatin – on lipoprotein oxidative functionality remains insufficiently characterized in subjects without established CVD. This study investigates the antioxidative effects of rosuvastatin by evaluating LDL susceptibility to oxidation and HDL antioxidative potential in patients with metabolic risk factors without confirmed CVD.
Methods: Open-label, one-arm prospective clinical trial, 50 statin-naïve adult outpatients (mean age 54.1 ± 10.9 years; 32 female, 18 male) with hyper/dyslipidemia, owerveight or obese, and one third with diabetes type 2 were administered rosuvastatin (20 mg/day) over 12 weeks. Lipid profiles, inflammatory markers, and oxidative parameters were measured at baseline, post-treatment, and after discontinuation. LDL oxidation was assessed via conjugated dienes formation; HDL antioxidative capacity via the TRAP assay. Statistical analyses included t-tests, nonparametric tests, and multivariate logistic regression. Significance was defined as p ≤0.05.
Results: Rosuvastatin significantly decreased LDL susceptibility to oxidation in 67% of subjects (CD-max reduction, *p* = 0.03), while changes in baseline oxidation and lag time were not statistically significant. HDL antioxidative capacity improved in 53% of subjects, although without statistical significance (*p* = 0.07). Multivariate analyses identified lipid parameters, hemoglobin, and IL-1β/IL-8 levels as significant predictors of oxidative LDL indices. Male gender (OR = 9.35; *p* = 0.010) and greater reduction in cardiovascular relative risk (OR = 0.338; *p* = 0.027) were independently associated with improved HDL antioxidative potential.
Conclusion: Rosuvastatin exerts beneficial effects on LDL oxidative susceptibility, with variable influence on HDL functionality. These findings underscore the importance of integrated assessment of lipid and redox biomarkers in cardiovascular risk profiling, especially among low-to-moderate risk populations.

EFFECTS OF CLASSIC ANTI-ARRHYTHMIC DRUGS IN SHORT QT SYNDROME TYPE 5: PRECLINICAL INSIGHTS FROM HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Objective: This study aimed to establish a patient-specific human induced pluripotent stem cell (hiPSC) model of SQTS5 and to provide insights into its pharmacological modulation. Methods: hiPSCs carrying the CACNB2 variant (c.1439C > T; p.S480L) were generated from a symptomatic SQTS5 patient and differentiated into cardiomyocytes (hiPSC-CMs). Isogenic controls were created via CRISPR/Cas9-mediated mutation correction. Electrophysiological properties at the single-cell level were evaluated using patch-clamp and calcium transient as well as optical mapping assays.
Results: SQTS5-hiPSC-CMs showed shortened action potential duration (APD) and reduced L-type calcium channel current (ICa-L). Among tested drugs, only disopyramide and ibutilide significantly prolonged APD via enhancing ICa-L and late sodium channel current (late INa), and inhibiting rapid (IKr), slow (IKs) delayed rectifier potassium currents and the transient outward potassium current (I_{to}), respectively. Disopyramide also suppressed arrhythmia-like events. At 2D level, the SQTS5 phenotype was recapitulated, with shortened APD and wavelength but preserved conduction. Drug testing confirmed that both disopyramide and ibutilide prolonged APD and wavelength; however, only disopyramide fully restored wavelength to isogenic control levels.
Conclusions: This patient-specific hiPSCs recapitulates phenotypes of SQTS5 and enables drug testing. Disopyramide emerges as a candidate for potential therapeutic use in SQTS5.

ROLES AND MECHANISMS OF NLRP3 INFLAMMASOME RELATED SIGNALING IN PATHOGENESIS OF BRUGADA SYNDROME

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Background: Fever and inflammation can exacerbate the phenotype of Brugada syndrome (BrS). However, there is still a lack of experimental studies investigating the underlying mechanisms of fever or inflammation. NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome is activated during infections. This study aimed to study the potential roles of NLRP3 inflammasome in the effects of inflammation on the phenotype of BrS.
Method: The human induced pluripotent stem cell (hiPSC) lines were generated from fibroblasts of a BrS patient harboring a mutation (c.3148G>A, p.Ala1050Thr)) in SCN5A, as well as from a healthy donor and a site-corrected (using CRISPR/CAS9) cell line. The hiPSC cell lines were used for the differentiation of cardiomyocytes (hiPSC-CMs). qPCR analysis was conducted to measure the mRNA level of PKC subtype (PKC-a, PKC-b, and PKC-e) after treatment with LPS or LPS plus MCC950, an NLRP3 inflammasome inhibitor. Arrhythmic events in BrS-hiPSC-CMs and healthy or isogenic control were assessed by calcium transient analysis. Sodium channel currents (INa) were measured by patch clamp. Western Blotting was performed to measure SCN5A expression.
Result: LPS reduced peak INa and increased the number of cells showing arrhythmic events in BrS-hiPSC-CMs but not in healthy donor-hiPSC-CMs, implying that LPS can exacerbate BrS phenotype. In addition, effects of LPS were inhibited by the NLRP3 inflammasome inhibitor MCC950. Arrhythmic-like events in BrS-CM were inhibited by MCC950. NLRP3 activator and IL-18 could mimic LPS effects on INa in BrS-CM. LPS significantly increased the expression level of PKC subtypes including PKC-a, PKC-b and PKC-e in healthy donor-hiPSC-CMs and isogenic cells. Strikingly, the LPS effects on PKC level could be inhibited by MCC950 in healthy-, but not BrS-cells, suggesting an involvement of different signaling in BrS-cells.
Conclusion: The NLRP3 inflammasome may contribute to arrhythmogenesis relating to inflammation in BrS patients by reducing PKC-a, PKC-b, and PKC-e. The NLRP3 inflammasome may be a potential therapeutic target for BrS.

INVOLVEMENT OF CAV1.2 CHANNELS IN A LQT2 (HERG) MUTATION: ION CHANNEL CROSSTALK?

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We recently set out to investigate estrogen-sensitivity of the phenotype in hiPS-derived-cardiomyocytes (CMs) from a HERG (LQT2) mutation carrier with marked QT prolongation (QTc ≈ 500 ms). Estrogen's effects pointed to unexpected involvement of ICaL (CaV1,2 channel) in the mutation phenotype. ICaL gating, but not density, was strongly affected by the mutation, leading to augmentation of the current during the action potential plateau. All subunits of the CaV.12 channel were genetically normal and ICaL abnormalities were reversed by superfusion with estrogen, thus suggesting a functional nature of the abnormality. This leads to hypothesize an unforseen crosstalk between the mutant HERG channel and the CaV1.2 one, significantly contributing to the disease phenotype, with potential relevance for therapeutic indications. While the underlying mechanism is still under investigation, examples of functional crosstalk between different ion channels have been reported and will be briefly reviewed in the talk.

ROLES AND MECHANISMS OF LACTATE IN PATHOGENESIS OF TAKOTSUBO SYNDROME STUDYING IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Introduction: The exact mechanisms of Takotsubo syndrome (TTS) are so far unclear. The role of lactate in cardiovascular disease is gradually being recognized. This study is designed to explore roles and mechanisms of lactate in pathogenesis of TTS.
Method: Human induced pluripotent stem cell derived cardiomyocytes(hiPSC-CMs) generated from one healthy donor and one TTS-patient were used as a study platform. 100uM epinephrine (Epi) was applied to hiPSC-CMs

to mimic the TTS setting. The qPCR, western blot, ELISA, immunostaining and patch clamp, metabolomics analysis, transcriptomics analysis and Mitochondrial Oxygen Consumption Rate Detection were performed.

Results: Epi-treatment increased lactate levels in both healthy (control) and TTS-hiPSC-CMs, detected by ELISA. WB and immunofluorescence further confirmed that the Epi elevated the level of histone (H3K18LA) lactylation through the NF-KB pathway. By using lactate dehydrogenase inhibitor(10uM), a significant reduction in ROS levels was found. In the Epi and TTS groups, significant prolongation of action potentials and increased arrhythmic events were found, which were significantly alleviated by lactate dehydrogenase inhibitors. Transcriptome results showed that ASIC1a (an acid-sensitive ion channel type1a) gene was significantly increased in the Epi treated cells. WB and qPCR further verified the result. By measuring intracellular PH value and ASIC current, it was found that lactate activated ASIC current through reducing PH value.

Conclusion: Lactate plays important roles for arrhythmogenesis of TTS via affecting ASIC1a channel. Lactate blockers might be clinically helpful for treating arrhythmias in patients with TTS.