Danish Cardiovascular Academy

ABSTRACTS

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O_1: Diabetes and sudden cardiac death in a nationwide, unselected population

Authors: T. Skjelbred, TH. Lynge, B. Winkel, J. Tfelt-Hansen

Background and purpose: Sudden cardiac death (SCD) remains a prominent contributor of mortality, with individuals afflicted by diabetes bearing a heightened risk of ventricular arrhythmias and SCD. However, comprehensive investigations in a nationwide, unselected context are lacking. Therefore, this study aimed to compare the incidence rates (IR) of SCD among individuals with Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) to the general population.

Methods: The study included the entire Danish population (approximately 5.5 million individuals) in 2010. SCD cases were identified through the detailed Danish death certificates and discharge summaries, yielding 6851 cases of SCD. The Danish Register of Medicinal Product Statistics, containing comprehensive prescription data from Danish pharmacies, facilitated the identification of individuals with T1DM and T2DM. Loss of life years was estimated for both T1DM and T2DM patients.

Results: Among the identified 6851 SCD cases, 272 (4%) were diagnosed with T1DM, and 833 (12%) with T2DM. Incidence rates of SCD were consistently elevated across all age groups for individuals with diabetes, compared to the general population (Figure 1). Patients with T1DM exhibited higher rates than those with T2DM (Figure 1). Notably, individuals aged 30-40 years with DM showed the greatest differences in incidence rates of SCD compared to the general population, with incidence rate ratios of 20.0 (95% confidence interval: 11.8-80.0) and 5.6 (95% confidence interval: 2.7-14.0) for T1DM and T2DM, respectively. On average, each 30-year-old patient with either T1DM or T2DM experiences a reduction of 3.7 and 3 life-years, respectively, due to the increased risk of SCD in these groups (Figure 2).

Conclusion: This nationwide study examines the incidence rates of SCD among individuals with and without a history of DM across all age brackets. Notably, patients with diabetes display consistently elevated incidence rates across age groups, with a particularly pronounced risk observed in those with T1DM. For both T1DM and T2DM, SCD is an important contributing factor to their shorter life expectancy compared to the general population. The heightened risk among individuals with diabetes underscores the importance of improved risk stratification for these patients.

Figure 1 – Incidence of SCD by age groups and type of diabetes

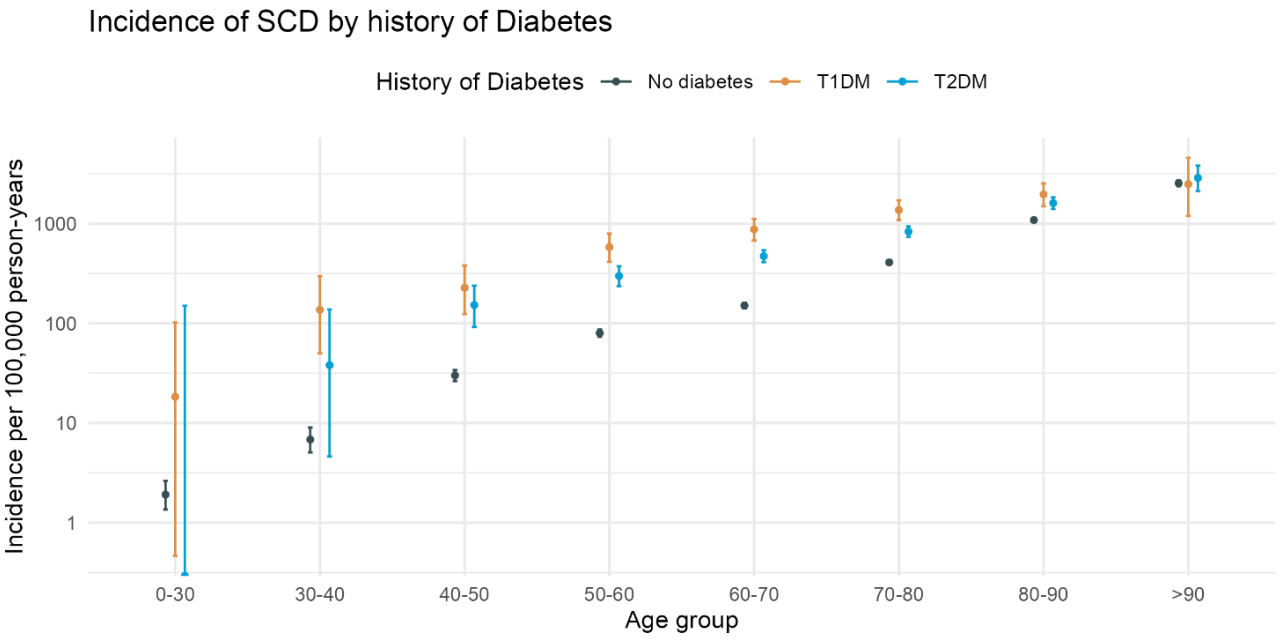
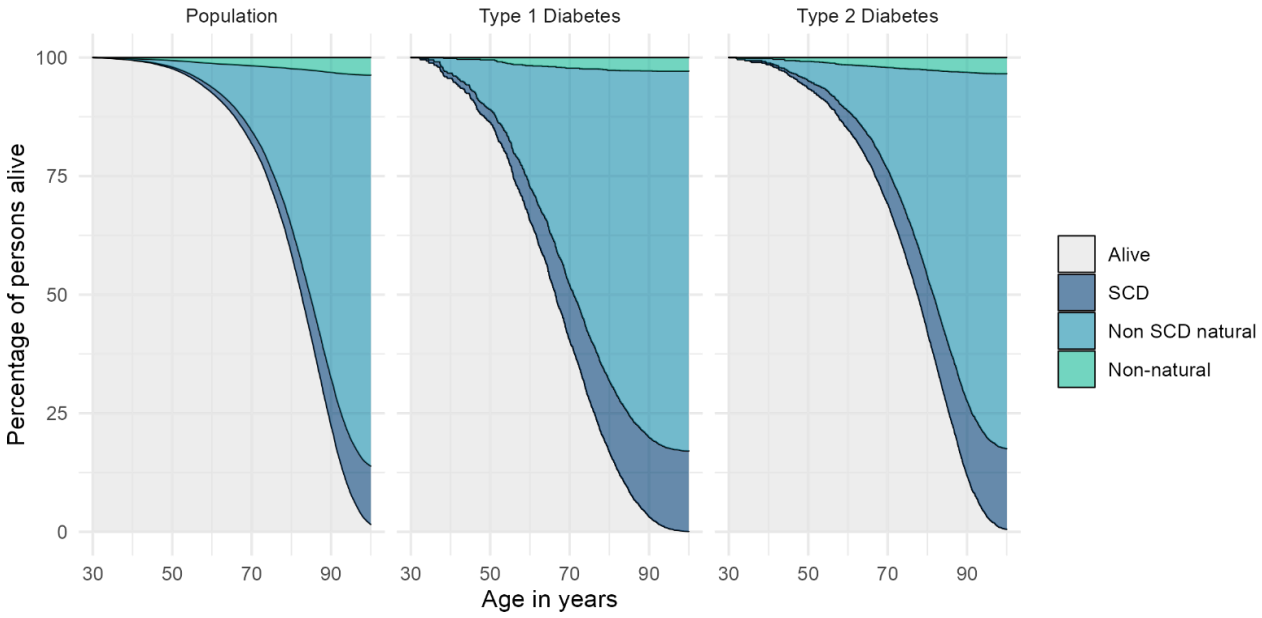


Figure 2 – Stacked cause-specific cumulative incidence curves for T1DM, T2DM and general population



O_2: Cardiac dysfunctions and the impact on clinical outcomes in patients with ST segment elevation myocardial infarction after percutaneous coronary interventions

Yan Zhou[1,2*], Jasmine Melissa Madsen[1], Lars Nepper Christensen[1], Kiril Aleksov Ahtarovski[1], Kasper Kyhl Kristensen[1], Christoffer Göransson[1], Lars Køber[1], Lene Holmvang[1], Frants Pedersen[1], Henning Kelbæk[1], Niels Vejlstrup[1], Jacob Thomsen Lønborg[1], Thomas Engstrøm[1]

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Aims The incidence and impact of cardiac dysfunctions after ST-elevation myocardial infarction (STEMI) are largely unknown. We aimed to characterize the prevalence of diastolic and systolic dysfunctions, stratified by peak filling rate/end-systolic volume (PFR/EDV) and left ventricular ejection fraction (LVEF), and to evaluate their effects on long-term outcomes in patients with STEMI after percutaneous coronary interventions (PCI).

Methods and results This is a sub-study of the DANAMI-3 trial including 425 patients with a cardiac magnetic resonance (CMR) during admission and three months after STEMI. PFR/EDV ≤ 2.55 and LVEF < 50% on CMR at three months were used to characterize cardiac dysfunctions. The outcome was the composite of all-cause mortality and hospitalization for heart failure. COX models were used to determine the hazard ratio (HR) for the outcome associated with dysfunction types. During a median follow-up of 10.5 years, there were 75 (17.6%) events of the outcome. The incidences of systolic dysfunction and no systolic dysfunction were 108 (25.4%) and 317 (74.6%). Adjusting for potential confounders, patients with systolic dysfunction were associated with higher risk of the endpoint than those without systolic dysfunction (adjusted HR 2.34, 95% CI 1.45–3.76). The incidences of diastolic dysfunction and no diastolic dysfunction were 178 (41.9%) and 247 (58.1%). Patients with diastolic dysfunction were not associated with elevated risk of the outcome. When patients were analyzed in four groups, the incidences of no dysfunction, isolated diastolic dysfunction, isolated systolic dysfunction, and both diastolic and systolic dysfunctions were 218 (51.3%), 99 (23.3%), 29 (6.8%) and 79 (18.6%). Isolated systolic dysfunction group was associated with increased risk of the endpoint (adjusted HR 3.63, 95% CI 1.77–7.48). Both diastolic and systolic dysfunction group was associated with increased risk of the endpoint (adjusted HR 1.90, 95% CI 1.07–3.39).

Conclusion Cardiac dysfunction was frequently observed in patients with STEMI treated with primary PCI. Systolic dysfunction with and without diastolic dysfunction was associated with increased clinical outcomes.

O_3: Quality of life after screening for atrial fibrillation – a LOOP substudy

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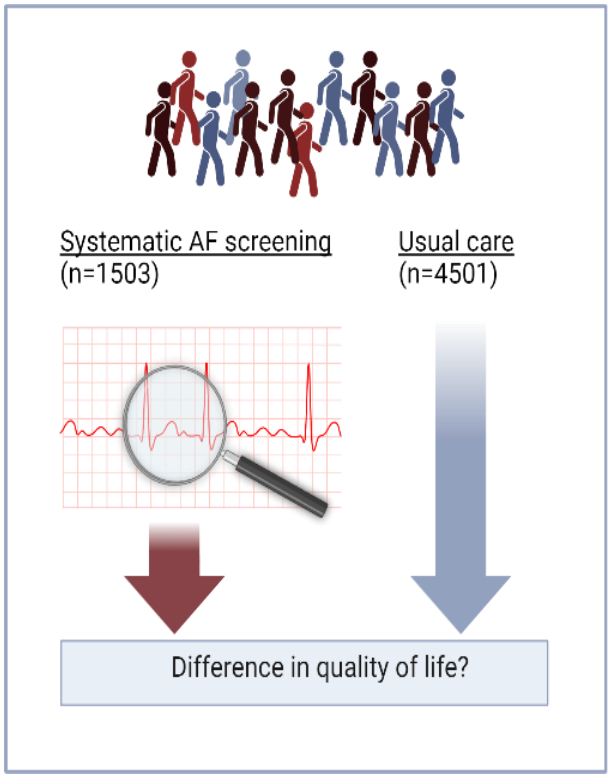
Background & Aim

Population screening for atrial fibrillation (AF) has not proven efficacious for stroke prevention. Nonetheless, screening is rising, and the adverse effects, including those on quality of life, are largely unknown. We investigated how three years of systematic AF screening affected quality of life compared to usual care.

Methods

We assessed quality of life data in 6,004 trial participants with stroke risk factors randomised to usual care (n=4,503) or AF screening using implantable loop recorder with anticoagulation upon AF detection (n=1,501). EQ-5D-5L assessments of five domains [mobility, selfcare, usual activities, pain/discomfort, anxiety/depression], each graded with a score from 1 to 5, were converted using standardised weights to a combined EQ- index score (worst=-0.76 best=1.00). EQ-index and EQ-VAS score (0=worst, 100=best) were analysed with linear mixed-effects models from baseline through year three. Logistic regressions analysed the odds of major problems overall and in each EQ-domain (defined as score ≥3) Sensitivity analyses with imputation of missing data were performed.

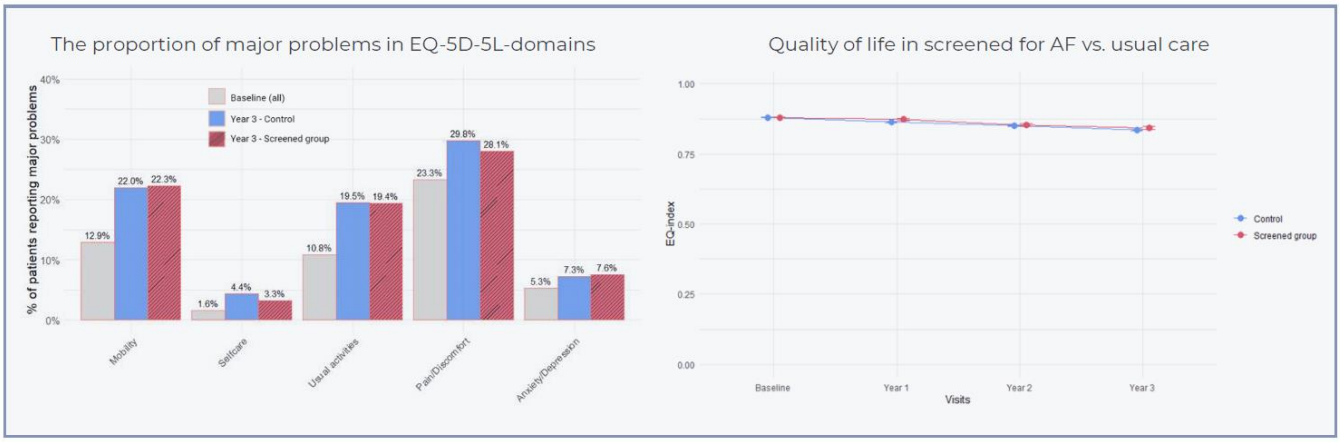


Results

Of 6,004 participants, 5,712 (95%) were alive after three years, and 5,162 (86%) had complete EQ-5D-5L data from baseline through year three. The mean age was 75 years (±4.1) and 47% were women. Common baseline diagnoses were hypertension (91%), diabetes (29%), and prior stroke, transient ischemic attack, or systemic arterial embolism (25%). The 5,712 participants who were eligible for three-year follow-up had a baseline EQ-index of 0.88 ±0.16, which had decreased by -0.05 (-0.05; -0.04) in the control group vs. -0.04 (-0.05; -0.03) in the screened group after three years, and baseline EQ-VAS of 78.4±16.2, which had decreased by -6.06 (-6.54; -5.57) in the control group vs. -5.26 (-6.43; -4.09) in the screened group after three years, with no significant difference between the groups (p=0.063 and p=0.18, respectively). The sensitivity analyses confirmed this. Major pain/discomfort problems were the most frequently reported problem throughout the study period, and the odds for any major problem did not differ between the groups after three years (odds ratio 0.91 (0.79;1.05)), which was confirmed by sensitivity analyses.

Conclusion

Systematic and intensive screening for AF had no impact on quality of life compared to usual care.



O_4: Comparative analysis of smooth muscle cell phenotypic diversity in atherosclerotic plaques across species and vascular beds

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Background:

Atherosclerotic plaques are primarily composed of smooth muscle cells (SMCs) and their progeny, which have lost their contractile function and acquired a diversity of phenotypes. Atherosclerosis is modeled using gene-modified animal models such as mice and pigs. However, it is unclear whether these animal models accurately represent the SMC phenotype diversity found in human atherosclerotic plaques, particularly between clinically significant human vascular beds and those typically studied in animal models.

Methods:

Using newly generated and publicly available single-cell RNA sequencing (scRNA-seq) data, we annotated diverse SMCs in atherosclerotic plaques from human carotid and coronary arteries, pig aorta and coronary arteries, and mouse brachiocephalic trunk. A unified SMC nomenclature was applied to facilitate dataset integration. Nine integration methods and three strategies of gene homology matching were employed to improve consensus identification of species-specific and shared SMC phenotypes. We validated and localized species- and vascular bed-specific SMC populations using RNAscope and immunofluorescent staining techniques.

Results:

The main SMC phenotypic axis varied from a contractile to a terminally modulated phenotype and was consistent across the studied species and vascular beds. However, several SMC populations were found to be species- or vascular bed-specific. We identified a human-specific SMC population, DLX5+ (distal-less homeobox 5 gene) SMCs, located in the medial layer of the carotid artery. Their transcriptional profile suggests involvement in neural crest formation pathways. Pericytes were found only in pig and human plaques across all studied vascular beds and localized to plaque neovessels. In human coronary plaque, two types of pericytes were found, only one of which was present in pig coronary plaques, which, however, contained a pig-specific population with a modulated proangiogenic (VEGFA+) phenotype. Mouse lesions contained SMC-derived cells with a chondrocyte-like phenotype, which were not present in pigs and humans. They were characterized by upregulation of cartilage-specific genes, such as Col2a1 (collagen type 2), and by RNAscope staining of Col2a1 in several atherosclerosis mouse models, they localized to areas of chondroid metaplasia in the plaque interior, often bordering necrotic core. Overall, pig plaques differed less from human plaques, suggesting that pigs may be needed to study some aspects of SMC diversity in atherosclerosis.

Conclusion:

Although SMC phenotypic diversity of atherosclerotic plaque varies in a species- and vascular bed-specific manner, the core SMC continuum remains stable across species and vascular beds.

O_5: Targeted proteome analysis reveals increased arterial basement membrane proteins and associations to glycemic status in patients with type 2 diabetes

Faarvang AA, Overgaard M, Jensen PS, Riber LPS, Wod M, Steffensen LBS, Rasmussen LM

Background: Type 2 diabetes mellitus (T2DM) is a major risk factor in the development of cardiovascular disease. However, the exact pathophysiological link is still unknown. In this study, we aim at quantitating the amounts of abundant proteins present in arterial tissue samples in individuals with type 2 diabetes mellitus. We hypothesize that the diabetic environment leads to an increase in arterial structural proteins such as collagen IV, which may in turn play a role in the increased arterial stiffening, build-up of arterial plaque and lower risk of aortic abdominal aneurysms seen in individuals with diabetes.

Patients and methods: We included 668 patients undergoing coronary artery bypass graft operation (CABG) at Odense University Hospital from 2008-2018. We utilize normal appearing arterial tissue samples from the internal thoracic artery. We quantitated 16 abundant cellular and extracellular proteins from formalin fixed paraffin embedded tissue, using targeted liquid chromatography tandem mass spectrometry (LC-MS/MS). Data were normalized to smooth muscle actin.
Results: Six proteins were increased among patients with diabetes: the four basement membrane proteins collagen IV, α1 (median protein ratio 1.54 vs. 1.35, p<0.001), collagen IV, α2 (median protein ratio 0.86 vs. 0.77, p<0.001), laminin (median protein ratio: 0.69 vs. 0.59, p<0.001), perlecan (0.78 vs. 0.72, p=0.002) as well as the two cytoskeletal proteins actin, cytoplasmic 1 (median protein ratio 4.46 vs. 4.27, p=0.002) and alpha actinin 4 (median protein ratio 1.66 vs 1.60, p=0.015). The amount of collagen IV and laminin was strongly correlated with HbA1c in the diabetic group (Pearson correlation coefficients above 0.3 (p<0.002).

Conclusion: In this study, we present quantitative amounts of tissue proteins from a large number of human non-atherosclerotic arterial tissue samples. We observed increased amounts in patients with T2DM of the basement membrane proteins collagen IV, α1 and α2, perlecan, laminin and of the cytoskeletal proteins alpha actinin 4, and actin, cytoplasmic 1. Collagen IV and laminin were strongly associated to HbA1c levels in the diabetic group. This upregulation may be associated with the increased arterial stiffness, dysfunctional remodeling and other cardiovascular pathologies seen in type 2 diabetes.

O_6: One-Year Risk of Heart Failure Following First Time Myocardial Infarction and Subsequent Mortality Risk: A nationwide study of 34,401 patients

Deewa Zahir, Daniel M Christensen, Mariam Malik, Nina Nouhravesh, Caroline Garred, Emil Fosbol, Lars Kober, Morten Schou

Introduction: A comprehensive understanding of the post-myocardial infarction (MI) trajectory leading to heart failure (HF) and its profound impact on long-term mortality is imperative for effective secondary prevention and risk assessment.

Methods: We conducted a registry-based nationwide cohort study of all Danish patients with a first MI during 2014 to 2020. Excluding patients with prior HF, individuals were followed for one year post-MI, assessing HF incidence through hospital admissions and visits to elective HF ambulatory clinics. Among those who survived one year, long-term mortality 1-6 years post-index MI was assessed using landmark analysis and Cox proportional hazards models adjusting for age, gender and comorbidities and hazard. Patients were categorized into three groups: not HF, referred for elective HF outpatient follow-up, and acute admission for HF.

Results: Among 34,301 individuals with a first MI and surviving the first year (median age 67.5, and 66.9 % male), 3.3 % were admitted acute for HF while 11.2 % had an elective HF outpatient follow-up within one year of the MI event. We observed a significant association between acute HF admissions post-MI and increased five-year mortality risk (hazard ratio [HR] 1.63, 95% confidence interval [CI] 1.40-1.90, p-value <0.001) (absolute 5-year risk: 18.4 %). In contrast, the five-year mortality risk for patients with elective outpatient HF follow-up was comparable to that of individuals without HF (HR 1.06 [0.94-1.19], p-value 0.34) (absolute 5-year risk: 9.6 vs 8.5 %).

Conclusion: Our findings suggest that acute admission for HF after a first time MI occur in 3.3 % of the patients with a subsequent significantly increased mortality risk compared to patients that do not develop clinical HF. Admission for HF in patients with a first time MI should be considered as a relatively rare but fatal complication.

Figure 1a: Cumulative incidence of all-cause mortality according to HF status 1 year post-MI

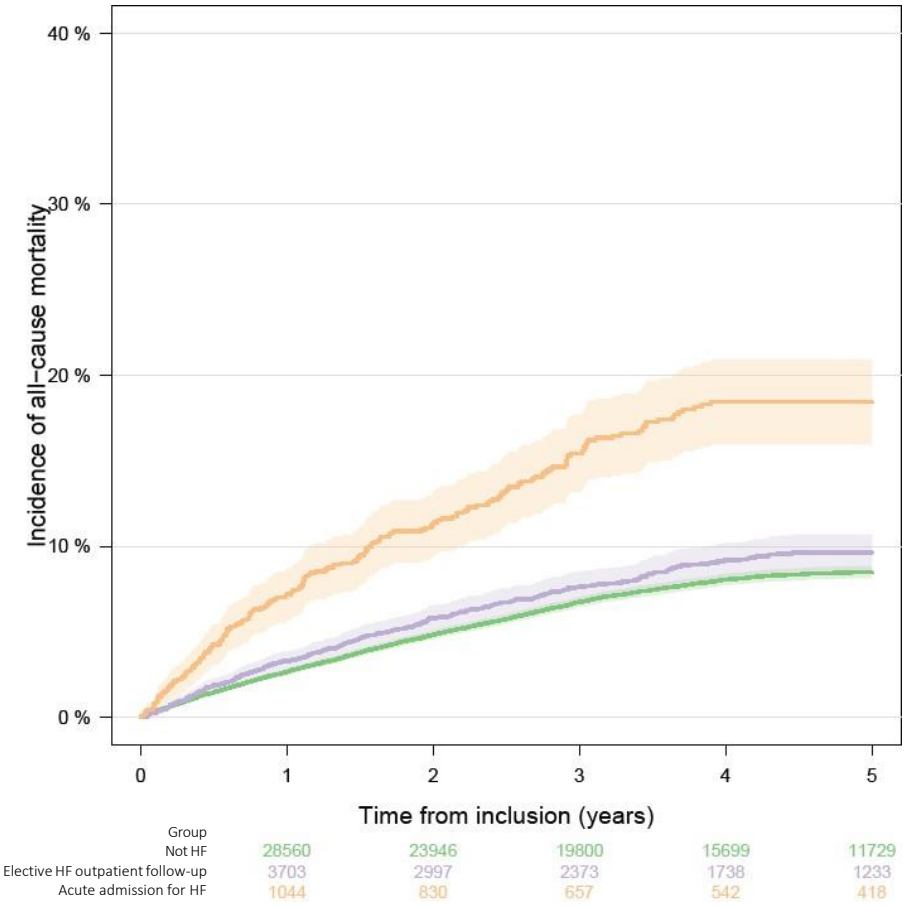
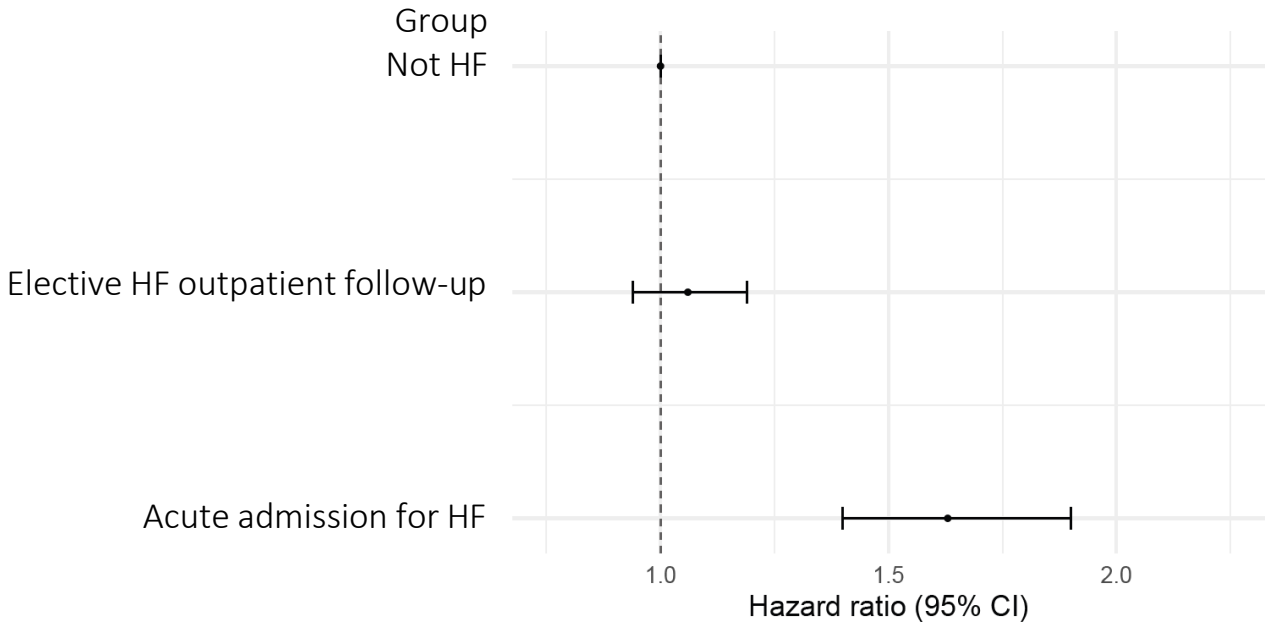


Figure 1b: Adjusted hazard ratios (HRs)* for risk of 5 year all-cause mortality according to HF status 1 year post-MI



O_7: NYHA functional class and ICD in non-ischemic heart failure with reduced ejection fraction: Extended follow-up of the DANISH trial

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Background: Current guidelines recommend implantable cardioverter-defibrillator (ICD) therapy in patients with heart failure (HF), a left ventricular ejection fraction (LVEF) of ≤35%, and New York Heart Association (NYHA) class II-III. While landmark primary prevention ICD trials have shown a clear and unequivocal benefit of ICD implantation in patients with NYHA class II, the evidence on the effect of an ICD in patients with NYHA class III appears to be less consistent.
Objectives: We investigated the long-term effects of primary prevention ICD implantation according to NYHA class in an extended follow-up study of the DANISH trial.
Methods: The DANISH trial randomized 1,116 patients with non-ischemic HF, a LVEF of ≤35%, NYHA class II-III (or IV if cardiac resynchronization therapy device implantation was planned), and elevated natriuretic peptide levels to ICD implantation or usual care. Outcomes were analyzed according to NYHA class at baseline (NYHA II and NYHA III/IV). The primary outcome was all- cause mortality.
Results: Of the 1,116 patients randomized in the DANISH trial, 597 (53.5%) were in NYHA class II at baseline, 505 (45.3%) in NYHA class III, and 14 (1.3%) in NYHA class IV. During a median follow-up of 9.5 years, ICD implantation, compared with usual care, did not reduce the long-term rate of all-cause mortality (all participants, HR 0.89 [95% CI, 0.74-1.08]; NYHA II, HR 0.85 [95% CI, 0.64-1.13]; NYHA III/IV, HR 0.89 [95% CI, 0.69-1.14]; Pinteraction=0.78) or cardiovascular death (all participants, HR 0.87 [95% CI, 0.74-1.08]; NYHA II, HR 0.78 [95% CI, 0.54-1.12]; NYHA III/IV, HR 0.89 [95% CI, 0.67-1.19]; Pinteraction=0.58), irrespective of NYHA class. Similarly, NYHA class did not modify the beneficial effects of ICD implantation on sudden cardiovascular death (all participants, HR 0.60 [95% CI, 0.40-0.92]; NYHA II, HR 0.73 [95% CI, 0.40-1.36]; NYHA III/IV, HR 0.52 [95% CI, 0.29-0.94]; Pinteraction=0.39).
Conclusions: In patients with non-ischemic HF with reduced ejection fraction, ICD implantation, compared with usual care, did not reduce the overall mortality rate, but it did reduce sudden cardiovascular death, regardless of baseline NYHA class.

Table. Effect of ICD implantation, compared with usual care, on outcomes according to NYHA class

Outcome	NYHA II N=597		NYHA III/IV N=519		P-value for interaction
	Control group N=300	ICD group N=297	Control group N=260	ICD group N=259	
Death from any cause					
N (%)	100 (33.3)	89 (30.0)	126 (48.5)	119 (45.9)	
Event rate per 100 person-years (95% CI)	4.3 (3.6-5.3)	3.8 (3.1-4.7)	7.0 (5.9-8.4)	6.4 (5.3-7.6)	
HR (95% CI)*	0.85 (0.64-1.13)		0.89 (0.69-1.14)		0.78
HR (95% CI)**	0.75 (0.56-1.00)		0.90 (0.70-1.16)		0.39
Cardiovascular death					
N (%)	67 (22.3)	55 (18.5)	97 (37.3)	92 (35.5)	
Event rate per 100 person-years (95% CI)	2.9 (2.3-3.7)	2.3 (1.8-3.0)	5.4 (4.4-6.6)	4.9 (4.0-6.0)	
HR (95% CI)*	0.78 (0.54-1.12)		0.89 (0.67-1.19)		0.58
HR (95% CI)**	0.68 (0.47-0.98)		0.91 (0.68-1.21)		0.30
Sudden cardiovascular death					
N (%)	24 (8.0)	18 (6.1)	33 (12.7)	17 (6.6)	
Event rate per 100 person-years (95% CI)	1.0 (0.7-1.5)	0.8 (0.5-1.2)	1.8 (1.3-2.6)	0.9 (0.6-1.5)	
HR (95% CI)*	0.73 (0.40-1.36)		0.52 (0.29-0.94)		0.39
HR (95% CI)**	0.69 (0.37-1.28)		0.52 (0.29-0.94)		0.44

CI, confidence interval; HR, hazard ratio; ICD, implantable cardioverter-defibrillator; NYHA, New York Heart Association.
**Stratified according to CRT status and center.*
***Stratified according to CRT status and center and adjusted for age and log of N-terminal pro-B-type natriuretic peptide.*

O_8: Human atherosclerotic plaques contain oxidant-modified proteins

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Compared to stable atherosclerotic plaques, unstable rupture-prone plaques contain higher levels of activated inflammatory cells that release the enzyme myeloperoxidase (MPO). MPO generates potent oxidants, including hypochlorous acid (HOCl), which may oxidize, chlorinate and nitrate amino acid residues and thereby damage extracellular matrix (ECM) proteins. Not only does such modification likely affect protein structure and function, it also results in altered cellular behavior of vascular cells seeded on damaged ECM, potentially contributing to plaque destabilization and rupture. As MPO is a source of oxidants, we hypothesized that MPO would also be a target for these species, and that human carotid artery atherosclerotic plaques would contain both oxidant-modified MPO and ECM proteins. The type, extent, and consequences of such modification remains unclear.

Proteins were extracted from unstable and stable human carotid artery plaques from patients with or without type 2 diabetes mellitus undergoing endarterectomy surgery. After digestion to peptides, proteins were subjected to mass spectrometry analysis to identify parent peptides and oxidation products. *In vitro* studies were carried out with purified human MPO.

More than 9000 proteins were identified from human carotid plaques, many of which were shown to contain oxidative modifications, including those generated by MPO. Our data indicates that modification (including chlorination and nitration of tyrosine (Tyr) residues) occurs on multiple ECM proteins, with differences in the pattern of these modifications detected between stable versus unstable plaques. Furthermore, these experiments indicate that 3 Tyr (Y203, Y343, Y459) and 2 tryptophan (W198, W255) residues within the MPO sequence can be chlorinated and/or nitrated *in vivo*. Purified human MPO exposed to its own oxidants was chlorinated at these residues in addition to multiple other sites.

In conclusion, human carotid plaques contain multiple oxidant-modified proteins. These modifications may alter protein structure and function, weakening plaque structure and enhancing their susceptibility to rupture.

O_9: Coronary computed tomography angiography prior to catheter ablation in patients with atrial fibrillation – the FIBCAG trial

Authors: Caroline Espersen, MD[1,2], Niklas Dyrby Johansen, MD[1,2], Kristian Eskesen, MD, PhD[3], Allan Zeeberg Iversen, MD, PhD[3], René H. Worck, MD, PhD[3], Martin H. Ruwald, MD, PhD[3], Morten Lock Hansen, MD, PhD[3], Raúl San José Estépar, MSc, PhD[4], Gregory M. Marcus, MD, MAS[5], Arne Johannessen, MD, DMSc[3], Jim Hansen, MD, DMSc[3], Tor Biering-Sørensen, MD, MPH, MSc, PhD[1,2]

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Background: Although catheter ablation (CA) is an effective treatment for atrial fibrillation (AF), recurrence rates remain high. Coronary artery disease (CAD) has been shown to be associated with AF development and recurrence. In this study, we seek to investigate whether screening for CAD on cardiac computed tomography (CT) prior to CA and initiation of appropriate treatment can improve CA outcomes.

Methods: In an investigator-initiated, prospective, single-center, randomized, open-label, controlled trial, we aim to include 852 adult patients with AF scheduled for a CA treatment at Copenhagen University Hospital Gentofte, Denmark. All patients undergo a cardiac CT scan including coronary CT angiography prior to CA. Patients are randomized 1:1 to either have the CT scan analyzed or not. In the intervention group, relevant anti-ischemic treatment and referral for further testing will be initiated based on the CT scan. After the CA, all patients will have an implantable cardiac monitor (ICM) implanted. All patients will be followed for at least 1 year.

Results: The primary outcome is the recurrence of atrial tachyarrhythmia lasting ≥30 seconds between 91 days after ablation and end of follow-up based on data from the ICM and electronic medical record. Secondary endpoints include hospitalizations for atrial tachyarrhythmias, acute coronary symptoms, stroke, and revascularizations.

Conclusion: This study will assess whether a screening strategy for CAD prior to CA can improve CA outcomes. The results will provide a better understanding of the implications of CAD in patients with AF undergoing CA and potentially guide clinical practice.

O_10: Maternal and Neonatal Characteristics Influencing Delayed Closure of Ductus Arteriosus: A CBH Cohort Study

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Background

The ductus arteriosus (DA), an essential part of fetal circulation, closes during the neonatal cardiac transition after birth. For unknown reasons, the DA closure is delayed in around 0.7% of term-born neonates. Utilizing the Copenhagen Baby Heart (CBH) Cohort, this study assesses maternal, prenatal, and perinatal factors associated with delayed closure in term-born neonates within the first 28 days after birth.

Method

In this study, we have analyzed data from full-term neonates who underwent echocardiography within 28 days after birth. The subjects were part of the CBH Study database, a population-based cohort exceeding 25,000 neonates. We excluded neonates with other congenital heart defects than atrial septal defects or patent foramen ovale. Exposures included maternal and neonatal factors. The primary outcome was an open DA based on echocardiography. We present effect estimates as adjusted risk ratios (aRR) with 95% confidence intervals (95%CI). Confounders were considered separately for each exposure.

Results

We included 21,610 neonates. We found the following factors associated with an open DA: female sex aRR=1.2 (95%CI: 1.0-1.4), hypoglycemia aRR=5.3 (95%CI: 2.1-12.8), African ethnicity aRR=2.8 (95%CI: 1.7-4.91) or Asian ethnicity aRR=1.9 (95%CI: 1.3-2.8), maternal obesity aRR=1.7 (95%CI: 1.1-2.6), and maternal type one diabetes aRR=2.6 (95%CI: 1.2-5.7).

Conclusion

We identified factors such as obesity and type one diabetes, neonatal hypoglycemia, and ethnicity as potential risk factors for delayed DA closure in term-born neonates. These findings contribute to understanding the ductus arteriosus and identifying at-risk groups early on to improve neonatal care and outcomes.

O_11: Glycosylated Atrial Natriuretic Peptide has the ability to increase renal blood flow in spontaneous hypertensive rats.

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*lead presenter

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Background.

Patients suffering from chronic kidney disease (CKD) has seen a steady increase in number over the last decades. However, only a few pharmaceutical substances have been approved for the condition during the last forty years.

Aim.

In order to investigate the effects on vasculature and potential renoprotective characteristics, we conducted a trial concerning infusion of a Atrial Natriuretic Peptide glycoform (gANP) in spontaneous hypertensive rats (SHR).

Methods

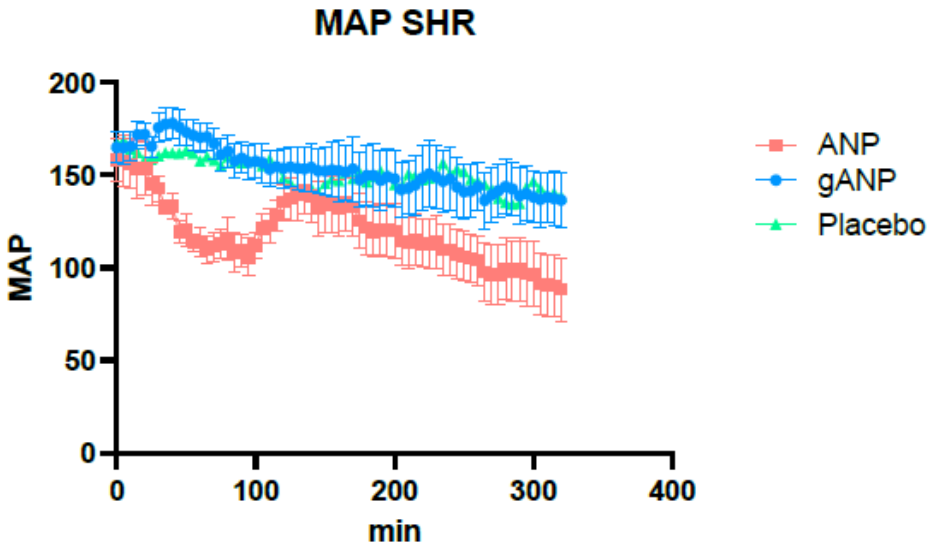
This study was conducted as a RCT including a total of eleven SHR, consisting of a one-hour infusion of either gANP, ANP or isotonic saline followed by four hours of observation. Blood pressure (BP), heart rate (HR) and doppler flow in left renal artery (RBF) was measured continuously, urine output was measured and collected every twenty minutes.

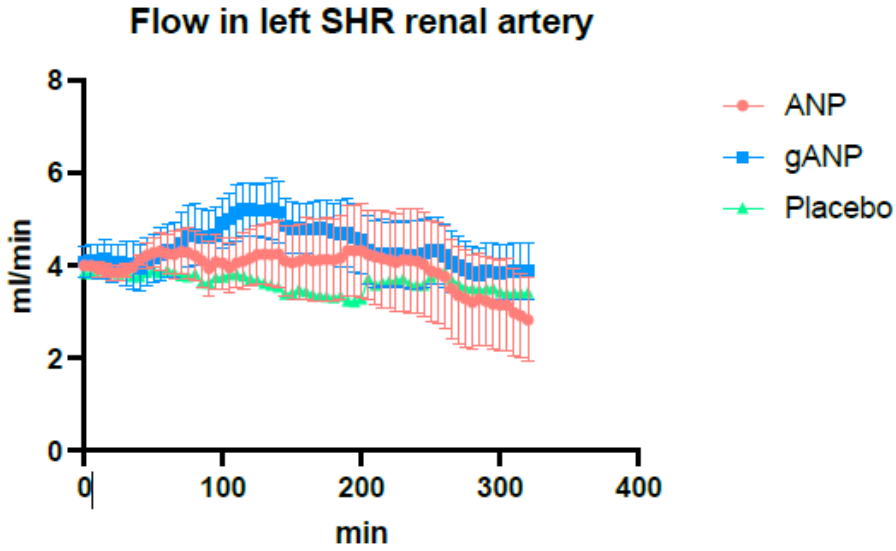
Results

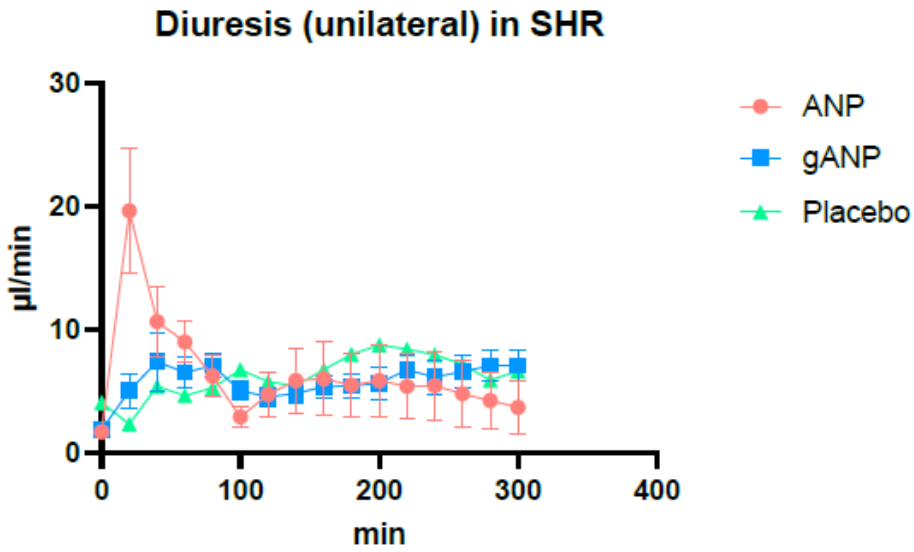
As opposed to ANP, infusion of gANP resulted in unchanged HR and BP yet a twenty percent increase of RBF was noticed. Furthermore, the effect persisted for several hours after infusion was completed and was not accompanied by an increase in diuresis.

Conclusion

Infusion of gANP led to an increase in renal blood flow without causing systemic hypotension in SHR. Accordingly, the vasoactive peptide in question shows potential for improved renal oxygen supply which possibly could delay a further decrease of glomerular filtration rate.


MAP SHR
MAP
200
150
100
50
0
0
100
200
300
400
min
ANP
gANP
Placebo


Flow in left SHR renal artery
ml/min
8
6
4
2
0
0
100
200
300
400
min
ANP
gANP
Placebo


Diuresis (unilateral) in SHR
µl/min
30
20
10
0
0
100
200
300
400
min
ANP
gANP
Placebo

O_12: Molecular Remodeling in Horse Atrial Fibrillation: The Effects of Metformin

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Introduction: Atrial Fibrillation (AF) is a common arrhythmia in both horses and humans, sustained by electrical, structural, and metabolic changes that compromise treatment efficacy. Here, we investigate the molecular remodeling associated with persistent AF and evaluate the potential of metformin, an indirect activator of AMP-activated protein kinase (AMPK), in preventing these changes.

Methods: Twenty retired racehorses underwent AF induction through two weeks of right atrial tachypacing, and were treated with either metformin (n = 10) or a placebo (n = 10). Additionally, four sham-operated controls were maintained in sinus rhythm. After four months, biopsies were collected from the right- (RA) and left atria (LA). We used data-independent acquisition mass spectrometry and RNA-sequencing on all samples (n = 48). Expression profiles were compared between groups to evaluate the effects of AF (placebo vs. sham) and metformin (metformin vs. placebo).

Results: When evaluating the effects of AF, we detected 13,801 transcripts, of which 1,880 genes differentially regulated in the RA and 725 in the LA. Gene-set enrichment analysis (GSEA) highlighted an upregulation of mitochondrial and glycolytic pathways, and a downregulation of angiogenesis and protein kinase activity. Metformin treatment was associated with differential regulation of 5,671 genes in the RA, but no significant changes were observed in the LA. On GSEA, genes related to angiogenesis and protein-phosphorylation were upregulated in the RA, including the AMPK-signalling pathway.

Conclusion: Four months of AF induced significant molecular changes across both atria, primarily affecting metabolic processes and protein phosphorylation. Metformin treatment appeared to mitigate some of these changes in the RA, potentially through AMPK-signaling.

O_13: Modulation of smooth muscle cell phenotype in atherosclerosis through SMAD7

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Background: In atherogenesis, smooth muscle cells (SMCs) lose their contractile phenotype, proliferate, and modulate to alternative phenotypes that drive plaque growth. However, it is unclear whether forcing the contractile phenotype represents a viable therapeutic target and what the most effective strategy would be to achieve that.

Hypothesis: We hypothesize that SMAD7, an inhibitory member of the SMAD family, is an important regulator of SMC phenotype in atherosclerosis and that its deletion may shift the balance of modulated SMCs towards contractile phenotype, preventing plaque growth.

Methods: *In vitro* functional assays and gene expression analysis were performed in siRNA-mediated SMAD7-knockdown rat aortic SMCs. In addition, atherosclerotic lesions were induced in a SMC lineage-traced and SMC-specific *Smad7* KO mouse (*Smad7*$^{\delta SMC\text{-}KO}$) by injection of rAAV-PCSK9^{D377Y} followed by 20 weeks of a high-fat diet. Histological analysis and plasma cholesterol analysis were used to assess the role of SMAD7 in atherosclerotic development and plaque composition.

Results: We showed that suppression of SMAD7 promotes the quiescent and contractile state of SMCs *in vitro*, suggesting that targeting SMAD7 can be used to force contractile phenotype in SMCs in atherosclerosis. *Smad7* deletion in SMCs in atherosclerotic mice lead to increased adipose tissue and enhanced lipid accumulation in the liver compared to WT littermates, with no significant changes in total body weight. In addition, plasma cholesterol levels were significantly higher in *Smad7*$^{\delta SMC\text{-}KO}$ atherosclerotic mice. Oil red O staining of *en face* aortas and Orcein staining of cross-sectioned aortic roots showed no differences in plaque lesion size between genotypes.

Conclusion: Our data suggests that targeting SMAD7 in SMCs affects lipid metabolism during atherogenesis. Despite increased circulating levels of cholesterol and richer lipid deposits in the liver, *Smad7*$^{\delta SMC\text{-}KO}$ mice do not develop larger lesions, which may indicate a protective role of *Smad7* deletion in plaque SMCs for atherosclerosis progression. Detailed analysis of structural and cellular composition of the lesions will help us to better understand the potential beneficial effect of targeting SMAD7 in plaque SMCs.

O_14: The role of ciliary genes in congenital heart disease comorbidities

Menachem Viktor Sarusie, Yeasmeen Ali, Daniel A. Baird, Søren Tvorup Christensen and Lars Allan Larsen

Congenital heart disease (CHD) is one of the most common birth defects worldwide and comprise simple to complex heart malformations. Despite significant improvements in treatment over the last 50 years, many CHD patients present with severe comorbidities, in particular neurodevelopmental disability (NDD). The aetiology of NDD in CHD patients is not known, but it has been suggested that it is caused by gene variants that alter both cardiac patterning and brain development. Genetic variants which impair cilia formation and function are known to cause severe rare human syndromes (ciliopathies) with overlapping phenotypes including CHD and NDD. In this project, we aim to identify cilia-related genes involved in the development of both heart and brain. We initially performed a meta- analysis of rare copy number variants from a large CHD cohort and identified 27 candidate genes with possible cilia-related function during heart and brain development. In order to select disease-causing candidates, we performed a crispant screening in zebrafish and characterized brain and heart development and function. This approach yielded five candidate genes (*ZMYND19*, *14-3-3ε/YWHAE*, *CSN1/GPS1*, *SEC5/EXOC2* and *GCP3/TUBGCP3*) and allowed us to further examine their localization at the primary cilia during *in vitro* cardiomyogenesis and neurogenesis.

Congenital heart disease (CHD) comprise simple to complex heart malformations which affects almost 1% of live births, making it one of the most common birth defects worldwide [1]. Ninety percent of CHD patients survive into adulthood due to significant improvements in treatment over the last 40-50 years and it is estimated that 12 million people are now living with CHD worldwide [2,3]. However, the treatment is not a cure, and recent research suggest that, for the majority of patients, CHD should be regarded as a life-long chronic disorder [2]. Adults with CHD have increased mortality [4], many patients present with severe comorbidities, in particular neurodevelopmental dissability (NDD) [5], and experience a progressive decline in cardiopulmonary function, which may lead to heart failure [2,6].
In most cases the association between CHD and NDD is unknown, but it has been suggested that NDD in CHD patients are caused by gene variants that alter both cardiac patterning and brain development [7,8]. Recently, our clinical collaborators have discovered that a large proportion of patients with simple forms of CHD also present with neurodevelopmental or psychiatric diagnosis, suggesting that brain-related comorbidities are more common among CHD patients than previously estimated [9-11]. Our group and others have shown that several signaling pathways, which control the development of the heart, is coordinated by the primary cilium [12-17]. Cilia are thin microtubule-based antenna-like organelles that project from the surface of most cells in the developing embryo, sending and receiving signals [18]. Genetic variants that impair cilia formation and function cause severe rare human syndromes (ciliopathies), with pleiotropic, overlapping phenotypes including CHD and NDD [12,19,20]. Thus, careful characterization of these genetic variants could lead to identification of novel genetic and molecular mechanisms that cause CHD and NDD. Such research can pave the way for early diagnosis, support and care for CHD patients with NDD co-morbidities, and provides an opportunity for identification of novel mechanisms involved in development and maturation of both human heart and brain.

O_15: Smooth muscle-specific deletion of Ccn2 causes severe aorta malformation and atherosclerosis

Larsen JH, *Hegelund JS, Pedersen MK, Andersson CM, Lindegaard CA, Hansen DR, Stubbe J, Lindholt JS, Hansen CS, Grentzmann A, Bloksgaard M, Jensen BL, Rodriguez-Díez RR, Ruiz-Ortega M, Albinsson S, Pasterkamp G, Mokry M, Leask A, Goldschmeding R, Pilecki B, Sorensen GL, Pyke C, Overgaard M, Beck HC, Ketelhuth DFJ, Rasmussen LM, *Steffensen LB*

Aims:

Cellular Communication Network Factor 2 (CCN2) is a matricellular protein implicated in fibrotic diseases, with ongoing clinical trials evaluating anti-CCN2-based therapies. By uncovering *CCN2* as abundantly expressed in non-diseased artery tissue, this study aimed to investigate the hypothesis that CCN2 plays a pivotal role in maintaining smooth muscle cell (SMC) phenotype and protection against atherosclerosis.

Methods and Results:

Global- and SMC-specific *Ccn2* knockout mouse models were employed to demonstrate that Ccn2 deficiency leads to SMC de-differentiation, medial thickening, and aorta elongation under normolipidemic conditions. Inducing hyperlipidemia in both models resulted in severe aorta malformation and a 17-fold increase in atherosclerosis formation. Lipid-rich lesions developed at sites of the vasculature typically protected from atherosclerosis-development by laminar blood flow, covering 90% of aortas, and extending to other vessels, including coronary arteries. Evaluation at earlier time points revealed medial lipid accumulation as a lesion-initiating event. Fluorescently labelled LDL injection followed by confocal microscopy showed increased LDL retention in the medial layer of *Ccn2* knockout aortas, likely attributed to marked proteoglycan enrichment of the medial extracellular matrix. Analyses leveraging data from the Athero-Express study cohort indicated relevance of CCN2 in established human lesions, as *CCN2* correlated with SMC marker transcripts across 654 transcriptomically profiled carotid plaques. These findings were substantiated through *in situ* hybridization showing *CCN2* expression predominantly in the fibrous cap.

Conclusions:

This study identifies CCN2 as a major constituent of the normal artery wall, critical in regulating SMC differentiation and aorta integrity, and possessing a protective role against atherosclerosis development. These findings caution against anti-CCN2-based therapies due to potential detrimental effects on the vasculature.

P1_1: Leadless or transvenous pacemakers for AV-block in elderly patients – the DANVERS trial protocol

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Background

Pacemaker implantation is the only treatment of high-grade atrioventricular block (AVB). While conventional transvenous pacemakers entail obvious clinical benefits for the patient, it is also associated with risk of complications, most notably risk of infection and lead-related complications. To reduce complication risk, leadless single chamber pacemakers have been introduced as an alternative to conventional pacing. Here, the entire system is enclosed in a single cylindrical unit implanted in the right ventricle. The leadless Medtronic Micra™ AV pacemaker can perform mechanical sensing of atrial contraction and pace the ventricles accordingly, so-called mechanical AV-synchronous VDD-pacing. No randomized trials have yet been conducted that directly compare leadless and transvenous dual-chamber pacemakers.

Methods

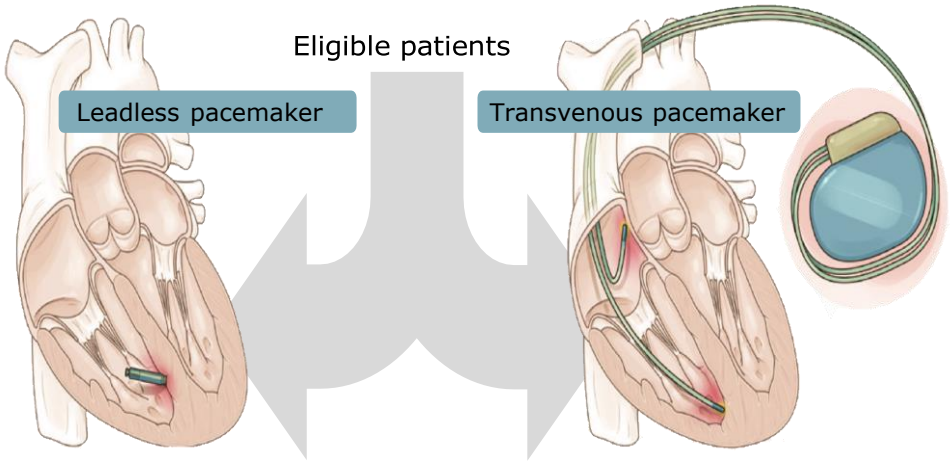
This study aims to evaluate quality-of-life with leadless and transvenous pacemakers in elderly patients with AVB. This is a multicentre investigator-initiated randomized combined parallel and cross-over trial. Patients with new-onset high-grade AVB and preserved sinus node function, aged 75 years or older, scheduled for first-time pacemaker implantation are eligible for inclusion. In the parallel part, patients are randomized 1:1 to implantation with the leadless Micra™ AV pacemaker or conventional transvenous pacemaker. In the cross- over part, all patients undergo three months of AV-synchronous VDD/DDD pacing and three months of non-AV-synchronous VVIR-pacing, to determine the importance of AV- synchrony. The primary endpoint is health-related quality-of-life measured by SF36 questionnaire.

Results

Inclusion is ongoing. 26/80 patients have been included. Follow-up is expected to be completed Summer 2025.

Conclusion

We expect this to be the first randomized trial to directly compare leadless and transvenous dual-chamber pacemakers.



P1_2: C-Reactive Protein and Long-Term Risk of Death in Patients with Myocardial Infarction

Julie Borchsenius[1], Kristian H. Kragholm[2], Martin Bødtker Mortensen[3], Alexander Egeberg[4], Christoffer Polcwiartek[2], Ditte Vesterlev[1], Christian Torp-Pedersen[5], Tor Biering-Sørensen[1], Manan Pareek[1]

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Introduction

The long-term prognostic value of C-reactive protein (CRP) in patients with myocardial infarction (MI) is unknown.

Purpose

To assess the association between the first CRP measured during hospitalization and long-term mortality in patients with MI.

Methods

Using Danish nationwide registries, we identified patients with a first diagnosis of MI from 2012 through 2020, who underwent a CRP measurement during index hospitalization. The primary outcome was death from any cause. The association between CRP levels and death was examined stratifying the patients into quartiles of CRP concentrations. Absolute and relative risks (RR) for death at days 0-30 and 31-365 were calculated through multivariable Cox regression with average treatment effect modeling. Models were standardized for demographic and clinical features, including high-sensitivity troponin (hsTn).

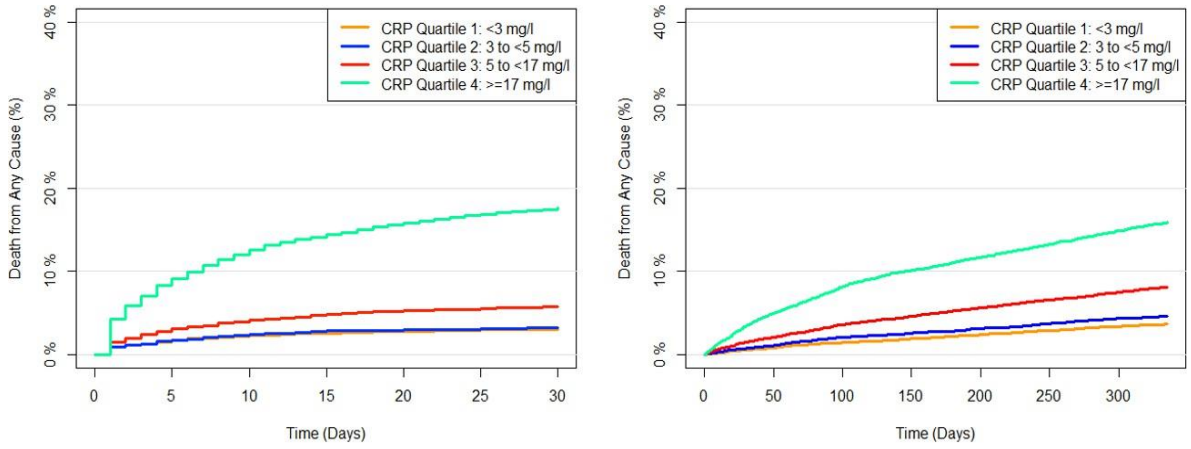
Results

We included 36,021 patients with MI and a CRP measurement within 24 hours before or after the time of admission. Median age was 69.7 years and 35.2 % were women. Median CRP in the entire cohort was 4.9 mg/l, and quartile (Q) intervals were: Q1: <3 mg/l, Q2: 3 to <5 mg/l, Q3: 5 to <17 mg/l, and Q4: 17 mg/l. CRP was significantly and nonlinearly associated with the primary outcome (p<0.001). At 0-30 days, 2694 patients had died, and another 2566 had died between days 31-365.

Figure 1 shows the Kaplan Meier curves. The standardized absolute risk of death at both 0-30 and 31- 365 days was lowest among patients in Q1 (0-30 days: 4.1%, 31-365 days: 4.8%) and highest among patients in Q4 (0-30 days: 12.1%, 31-365 days: 10.6%). The standardized RR of death compared with Q1 were: 0-30 days 1.01 (95% CI 0.84;1.71), 1.45 (1.26;1.63) and 2.96 (2.61;3.29) in Q2, Q3 and Q4 and at days 31-365 the RR were 1.18 (1.02;1.34), 1.61 (1.42;1.79) and 2.19 (1.95;2.42), respectively.

Conclusion

In patients with MI, higher CRP levels were significantly associated with a higher risk of death, independently of hsTn concentrations.



P1_3: N-Terminal Pro-B-Type Natriuretic Peptide Levels Pre-Transcatheter Aortic Valve Implantation and Relationship with Long-term Outcomes

Louise Marqvard Sørensen, MD;[1] Jeppe Kofoed Petersen, MB;[1] Jarl Emanuel Strange, MD, PhD;[1] Lauge Østergaard, MD, PhD[1]; Jacob Eifer Møller, MD, PhD[1,3]; Morten Schou, MD, PhD[2]; Lars Køber, MD, DMSc[1]; Ole de Backer, MD[1], PhD; Emil Fosbøl, MD, PhD[1]

Background: Blood levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) has been suggested as a future guidance tool for the selection of patients for aortic valve replacement. This study aimed to examine how levels of NT-proBNP pre-transcatheter aortic valve implantation (TAVI) is associated with one-year rates of heart failure (HF) admission and mortality following TAVI.

Methods: With Danish nationwide registries, we identified all patients undergoing TAVI from 2014–2021 who had at least one recorded NT-pro-BNP measurement within one year before TAVI. Patients were compared by quartiles of pre-TAVI NT-proBNP: quartile 4 (high NT-proBNP group) vs quartile 1-3 (low NT-proBNP group). Comparisons of all-cause mortality and HF-admissions were conducted using Kaplan-Meier analysis, cumulative incidence, and Cox analysis, as appropriate.

Results: We identified 1,140 patients undergoing first-time TAVI with a recorded NT-pro-BNP; 846 (74.2%) with a low NT-proBNP (< 420 pmol/L) (55.0% male, median age 81 year) and 294 (25.8%) with a high NT-proBNP (≥ 420 pmol/L) (53.1% male, median age 82 year). A high versus low NT-proBNP was associated with increased one-year cumulative incidence of HF-admissions (9.1% vs. 23.1%, adjusted HR 2.00 [95% CI, 1.33-2.74]) and all-cause mortality (6.0% vs. 14.6%, adjusted HR 1.95 [95% CI: 1.24-3.07]). A high NT-proBNP was associated with higher rates of outcomes irrespective of previously known atrial fibrillation, HF, chronic kidney disease, and hypertension.

Conclusion: In patients undergoing TAVI, a baseline NT-proBNP ≥420 pmol/L was associated with increased one-year rates of HF-admission and mortality post-TAVI and may be utilized to identify a high-risk population.

P1_4: The association between parents' social status and cardiovascular health outcomes in children.

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Background: Risk factors for cardiovascular disease, including childhood obesity, have been shown to accumulate in people with lower socioeconomic position (SEP). No previous study has examined the association between parent SEP and risk factors in children for future CVD, regardless of childhood obesity, including potential mechanisms.

Aim: We assess the association between parents' SEP and the current risk for developing CVD, as well as potential mechanisms behind the association, in children with obesity.

Methods: A total of 3455 children and adolescents were included from an obesity cohort in a cross-sectional study design. Data on anthropometric measures, blood pressure, lipid-status, body composition, smoking status, inflammation status, ANS activity and parent's educational and occupational status was collected. Descriptive statistics, univariate analysis, multivariate linear and logistic regression were applied to analyze the data.

Results: Compared to the children and adolescents belonging to social class 1, the children and adolescents in social class 3 showed a higher prevalence of greater weight, waist and hip measurements, BMI z-score, blood pressure (systolic and diastolic), active smokers (OR: 2.77), total fat mass and fat percent ($p<0.05$) Preliminary results for ANS activity and inflammation will also be presented.

Conclusion: Parent's SEP is associated to risk factors for CVD in later life in children and adolescents with obesity. Our study emphasizes the need for preventive care to be made during childhood and indicates focusing on approaching different social classes differently – perhaps taking possible mechanisms behind the association into account.

P1_6: Periostin as a biomarker for predicting atrial fibrillation

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Background & aim: Atrial fibrillation (AF) is the most common arrhythmia in humans, affecting 59 million people worldwide, and it is associated with a higher risk of stroke and heart failure. It is at the moment difficult to predict AF since initial symptoms are mild and passing. It is only later in the course of the disease, when the symptoms are more prominent, that treatment is initiated. These challenges highlight the importance of researching the underlying mechanisms leading to AF, to start treatment earlier and improve quality of life. Studies show that the hearts of AF-patients are characterized by remodeling of the tissue and with the use of biomarkers this remodeling can be examined. From preliminary results the biomarker periostin is suggested as a new existing marker for structural remodeling in the heart. The project aims to examine if periostin levels in heart biopsies and blood samples from patients undergoing open heart surgery, can help predict AF.

Materials & method: Heart biopsies and blood samples from 14 patients were included in the study and grouped according to whether the patients developed post-operative AF (POAF) or stayed in sinus rhythm (SR) (POAF: n=8, SR: n=6). Heart biopsies from right atrial appendages (RAA) were included in a Western blot analysis and the blood samples, collected before the surgery, were examined in a sandwich ELISA. A correlation calculation between biopsies from RAA and blood samples is to be performed with the hypothesis that blood analysis alone can help predict AF though more time-effective and less invasive analysis compared to biopsies analysis.

Results: Heart biopsies from RAA showed similar levels of periostin when comparing SR to POAF patients (POAF: 1.43±0.50, SR: 1.00±0.44, p=0.14). Results from the ELISA showed that serum samples from POAF and SR patients have similar levels of periostin (POAF: 1725±148 pg/mL, SR: 1728±227 pg/mL, p=0.99).

Conclusion: Periostin levels are not significantly different when comparing POAF patients to SR, both in blood samples and biopsies from RAA. Further categorization of the patients beyond the current SR and POAF groups may yield valuable insight into the underlying factors, like fibrosis, contributing to the development of AF and help us understand the mechanisms that predispose certain individuals to this condition.

Keywords: atrial fibrillation; atrial fibrosis; biomarkers; periostin; human

P2_1: A carrier-based quantitative proteomics method applied to biomarker discovery in pericardial fluid

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Abstract

Data-dependent liquid chromatography tandem mass spectrometry (LC-MS/MS) is challenged by the large concentration range of proteins in plasma and related fluids. We adapted the SCoPE method from single-cell proteomics to pericardial fluid, where a myocardial tissue carrier was used to aid protein quantification. The carrier proteome and patient samples were labeled with distinct isobaric labels, which allowed separate quantification. Undepleted pericardial fluid from patients with type 2 diabetes mellitus and/or heart failure undergoing heart surgery was analyzed with either a traditional LC- MS/MS method or with the carrier proteome. In total, 1398 proteins were quantified with a carrier, compared to 265 without, and a higher proportion of these proteins were of myocardial origin. The number of differentially expressed proteins also increased nearly four-fold. For patients with both heart failure and type 2 diabetes mellitus, pathway analysis of upregulated proteins demonstrated enrichment of immune activation, blood coagulation, and stress pathways. Overall, our work demonstrates the applicability of a carrier for enhanced protein quantification in challenging biological matrices such as pericardial fluid, with potential applications for biomarker discovery. Mass spectrometry data are available via ProteomeXchange with identifier PXD052067.

P2_2: Sodium-Glucose Cotransporter-2 Inhibition Prevents Development of Left Ventricular Hypertrophy

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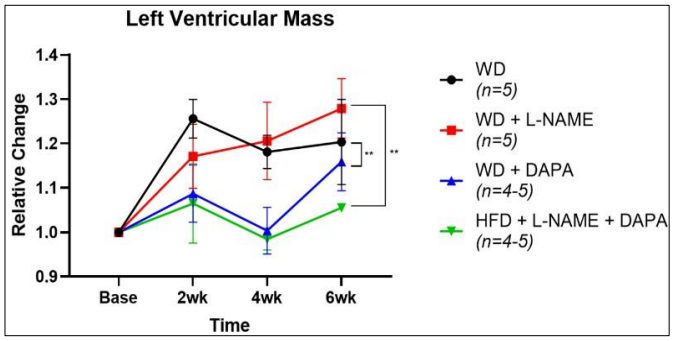
Introduction: Several clinical studies have demonstrated the significant beneficial effects of sodium-glucose cotransporter-2 (SGLT2) inhibitors in patients with heart failure regardless of diabetic status (1). The underlying mechanisms are unknown, but a recent study proposes that improved metabolic communication in the kidney by SGLT2-inhibition may play an important role (2). However, heart morphology and function were not investigated. Nevertheless, changes in cardiac metabolism are suggested to promote heart failure development, including left ventricular hypertrophy (3).

We hypothesized that SGLT2 inhibitors by preventing cardiac remodeling due to an improved cardiometabolic state may reduce severity of heart failure.

Methods: C57Bl/6jRj male mice were divided into these 5 subgroups; I) mice on a normal diet, II) vehicle-treated mice on Western Diet (WD); III) vehicle-treated mice on WD receiving N-nitro-1-arginine methyl ester (L-NAME, 600 mg/kg/day) in drinking water; IV) SGLT2 inhibitor-treated (dapagliflozin; 10 mg/kg diet (DAPA)) mice on WD; and V) SGLT2 inhibitor-treated mice on WD and L-NAME. The combination of WD and L-NAME is known to induce heart failure with preserved ejection fraction (4). All interventions lasted for 6-8 weeks.

Blood pressure measurements and echocardiography were conducted before intervention and every 2nd week during the intervention period. Data were analyzed using mixed-effects model or three-way ANOVA (GraphPad Prism 10.2.1) and are presented as means ± standard errors of the means. P<0.05 is considered statistically significant.

Results: L-NAME increased mean arterial pressure (MAP) in both groups receiving WD and WD+DAPA. Six weeks after intervention, MAP was 90±3, 115±4, 88±4 and 118±4 mmHg (n=6-8, *P*=0.0001) in WD, WD+L-NAME, WD+DAPA and WD+DAPA+L-NAME, respectively. L-NAME increased heart rate in the WD-group (587±13 BPM), but the effect was absent in the WD+DAPA-group (535±14 BPM).



No difference was observed between the groups in stroke volume, ejection fraction, fractional shortening, and cardiac output. However, left ventricular mass was increased in the WD- and WD+L-NAME-groups but prevented in both groups receiving DAPA (**, P<0.005).

Conclusion: Our results suggest that an underlying mechanism of cardiac protection by SGLT2-inhibitors may be prevention of left ventricular hypertrophy, which is a known part in the pathogenesis of heart failure.

P2_3: Using zebrafish to understand how mutations in desmosomal components contribute to the development of atrial fibrillation.

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Desmosomal components are essential for the propagation of electrical and mechanical intracellular signals in myocardial tissue. Mutations in desmosomal components are known to be a major cause of arrhythmogenic cardiomyopathy and recent studies suggest that mutations in desmosomal genes can also be associated with early-onset cases of AF. Using zebrafish models, our studies propose to characterize the involvement of desmosomal proteins in early- stage AF. In this study we focused on three desmosomal genes, *PKP2*, *JUP* and *DSC2*. Using CRISPR-Cas9 technology we generated mutant lines for *pkp2, jupa, jupb* and *dsc2l*, targeting regions where AF mutations have been identified in patients. Future studies will investigate the effect of the mutations on desmosome structure and describe changes in heat chamber morphology, volume, and heart calcium dynamics. Our results aim to enrich the understanding regarding the effect of the desmosomal mutations in AF. Moreover, our models are proposed to be used to further investigate new pharmacological targets for AF patients.

P2_4: Mouse model of heart failure with preserved ejection fraction

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Background: Underlying causes for heart failure with preserved ejection fraction (HFpEF) are not fully understood due to the complex pathophysiology of the disease. The patients often have several chronic co-morbidities such as hypertension, diabetes and obesity. Treatment options for patients with HFpEF are very limited, possibly because we do not understand the disease mechanism. We need translational animal models that accurately mimics the cardiovascular phenotype of HFpEF to study the pathological mechanisms and test new potential treatments.

Aim: The aim of this study is to develop and validate a mouse model of HFpEF.

Methods: C57BL/6N male mice at 8 weeks of age were fed a high fat diet (60% fat), and given L-NAME (NO-synthase antagonist; 1 g/L) in their drinking water, to induce obesity and hypertension, respectively. The treatment group was compared to an untreated age- matched control group. After 16 weeks of treatment, cardiac function was evaluated in vivo by echocardiography and ex vivo by an isolated heart perfusion technique called working heart.

Results: After 16 weeks of treatment, the HFpEF group presented with preserved ejection fraction, a reduction in cardiac index (cardiac output normalized to body surface area), and left ventricular hypertrophy. Diastolic dysfunction in the HFpEF group was evident by prolonged isovolumic relaxation time and elevated E/e' ratio compared to control group.
Conclusion: Based on the results from the cardiac ultrasound and working heart, current mouse model presents with a cardiovascular phenotype of HFpEF.

P2_5: In-Depth Phosphoproteomic Profiling of the Insulin Signaling Response in Heart Tissue and Cardiomyocytes Unveils Canonical and Specialized Regulation

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Background: Insulin signaling regulates cardiac substrate utilization and is implicated in physiological adaptations of the heart. Alterations in the signaling response within the heart are believed to contribute to pathological conditions such as type-2 diabetes and heart failure. While extensively investigated in several metabolic organs using phosphoproteomic strategies, the signaling response elicited in cardiac tissue in general, and specifically in the specialized cardiomyocyte cells, has not yet been investigated to the same extent.

Methods: Insulin or vehicle was administered to C57BL6/JRj male mice, and ventricular tissue was analyzed by quantitative phosphoproteomics to evaluate the insulin signaling response. Cardiomyocytes from a cardiac and skeletal muscle-specific *Tbc1d4* knockout mouse and wildtype littermates were used to study Tbc1d4's role in insulin signal transduction. Phosphoproteomic studies involved isobaric peptide labeling with Tandem Mass Tags (TMT), enrichment for phosphorylated peptides, fractionation via micro-flow reverse-phase liquid chromatography, and high-resolution mass spectrometry measurement.

Results: We quantified 10,399 phosphorylated peptides from ventricular tissue and 12,739 from isolated cardiomyocytes, localizing to 3,232 and 3,128 unique proteins, respectively. In cardiac tissue, we identified 84 insulin-regulated phosphorylation events, including sites on the Insulin Receptor ($Insr^{Y1351, Y1175, Y1179, Y1180}$) itself as well as the Insulin receptor substrate protein 1 ($Irs1^{S522, S526}$). Predicted kinases with increased activity in response to insulin stimulation included Rps6kb1, Akt1 and Mtor. Tbc1d4 emerged as a major phosphorylation target in cardiomyocytes. Despite limited impact on the global phosphorylation landscape, Tbc1d4 deficiency in cardiomyocytes attenuated insulin-induced Glut4 translocation and induced protein remodeling. We observed 15 proteins significantly regulated upon knockout of *Tbc1d4*. While Glut4 exhibited decreased protein abundances consequent to Tbc1d4- deficiency, Txnip levels were notably increased. Stimulation of wildtype cardiomyocytes with insulin led to the upregulation of 262 significant phosphorylation events, predicted to be regulated by kinases such as Akt1, Mtor, Akt2, and Insr. In cardiomyocytes, the canonical insulin signaling response is elicited in addition to regulation on specialized cardiomyocyte proteins, such as $Kcnj11^{Y12}$ and Dsp^{S2597}.

Conclusion: We present a first global outline of insulin induced phosphorylation signaling response in heart tissue and in isolated adult cardiomyocytes detailing the specific residues with changed phosphorylation abundances. Our study marks an important step towards understanding the role of insulin signaling in cardiac diseases linked to insulin resistance.

P2_6: Improved heart function by medium-chain triacylglycerol intake is associated to increased fat and ketone body availability

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Background and aim
In heart failure, cardiac mitochondrial fat oxidation is impaired. This seems related to impaired mitochondrial uptake of long-chain fatty acid via the carnitine system, increasing the reliance on circulating ketone bodies (KB). Accordingly, increased KB availability is suggested to improve cardiac function. Medium-chain triacylglycerol (MCT) have the ability to induce ketogenesis and increase whole-body fat oxidation independently of the carnitine system. Therefore, *the aims* of this study were to investigate the acute effects of MCT intake on 1) KB levels and lipid species and substrate metabolism, 2) cardiac function in patients with heart failure with reduced ejection fraction (HFrEF).

Methods
Study 1: 4 young, lean, healthy men were randomized in a crossover study to acute intake of 35g MCT or long-chain triacylglycerol (LCT) to investigate the responses in circulating KB and lipid species assessed by plasma lipidomic analysis and whole-body metabolism. Study 2: Participants with HFrEF and healthy matched participants ingested 45g MCT or LCT to investigate the acute effects on heart function assessed by cardiac magnetic resonance imaging.

Results
Acute intake of MCT increased blood KB to 0.9 and 1.4mmol/l in study 1 and 2. Lipidomic analysis revealed increased abundance of medium-chain fatty acids (MCFA) in plasma. Acute intake of MCT increased energy expenditure and fatty acid oxidation rate more compared with LCT. Intake of MCT increased cardiac output (CO) by 9.3±4.3 % and left ventricular ejection fraction (LVEF) by 4.3±1.9 % in healthy participants, and CO by 16.0±6.5 % and LVEF by 10.9±3.4% in participants with HFrEF relative to LCT intake.

Conclusion
Acute MCT intake increased circulating levels of ketone bodies and MCFAs, and whole-body fatty acid oxidation, potentially leading to the increase in CO and LVEF.

P2_7: Anti-inflammatory actions of lactate-induced HCA1 receptor activation and their consequences for cardiac ischemic damage and heart failure

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Ischemic heart disease - along with its common complications, heart failure (HF) and arrhythmia- continues to be a major cause of death and morbidity worldwide. Whereas the acute treatment of myocardial infarction (MI) has improved substantially over the past decades, the remodeling phase following MI remains detrimental for the development of HF and less well understood, illustrating the unmet medical need for new approaches to HF prevention and therapy.

Altered myocardial metabolism and local inflammation play key roles in cardiac wound healing. Several studies show that accumulating metabolites such as lactate serve roles as metabolic fuels in the failing heart and as important signaling molecules during ischemia. The G-protein coupled receptor HCA1 senses interstitial lactate and expression of HCA1 in the cardiovascular system and on immune cells underscores the potential physiological and pathophysiological relevance during cardiovascular disease development. Lactate-induced activation of HCA1 inhibits lipolysis and modulates local immune responses following acute organ injury. However, the role of HCA1 activation in the ischemic heart remains unresolved.

We now hypothesize that activation of HCA1 by lactate limits cardiac ischemic damage and improves cardiac remodeling following myocardial function. Using a transgenic mouse model with disrupted HCA1 expression we gathered baseline measurements of body composition (echoMRI), blood pressure (tail-cuff), and cardiac function (echocardiography). We induced ischemic HF in HCA1 knockout (KO) and wild type (WT) mice by ultrasound-guided electrocauterization of the left anterior descending artery (LAD). The mice were randomized to cages with running wheels or dummy wheels for 28 days. On day 25-27 we repeated blood pressure measurements and on day 28 we repeated body composition measurements and echocardiography. Finally, the animals were euthanized, and tissue was harvested for subsequent analyses.

Next, we will perform immunohistochemical tests on heart and skeletal muscle to assess muscle cell density, fibrosis, and investigate capillary density and immune infiltration. We will mount HCA1 KO and WT hearts in Langendorff setups to perform ischemia and reperfusion experiments ex vivo and assess infarct sizes by triphenyl tetrazolium chloride (TTC) staining.

Based on our hypothesis, we expect that activation of HCA1 by lactate will limit immune cell infiltration in the heart and cause a shift towards a cardioprotective, anti-inflammatory immune phenotype. Consequently, we expect that MI and HF following LAD obstruction will be more severe in HCA1 KO than WT mice.

P2_8: Ventricular repolarization and tachyarrhythmias in hibernating brown bears

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Background: Ventricular tachyarrhythmias, such as ventricular tachycardia (VT) and ventricular fibrillation (VF), are leading causes of sudden cardiac death. While hypothermia and hypokalemia are associated with ventricular arrhythmias in humans, brown bears rarely die during hibernation despite reduced serum potassium and body temperatures dropping to 32°C, suggesting protective adaptation in their electrophysiology. We intended to study the electro- cardiographic characteristics of ventricular tachyarrhythmias in brown bears (*Ursus arctos*).

Methods: We collected one-year recordings from 57 bears implanted with a loop recorder (Reveal XT, Medtronic, USA) between 2012 and 2019, from which we extracted electrocardiograms (ECG) from detected arrhythmic episodes for retrospective analysis. In addition, we extracted sinus rhythm (SR) recordings from six bears for control ECG analysis.

Results: Two episodes of self-terminating VT occurred during hibernation in one bear, lasting 16.6 and 21.3 seconds with rates of 170 and 166 BPM. The episodes were polymorphic in appearance, and coupling intervals were 523 and 351 ms, respectively. Initiation was characterized by a premature ventricular contraction (PVC) at the timepoint of the preceding T-wave (R-on-T phenomenon). The control SR recordings showed longer QT intervals and T_{peak}-T_{end} during hibernation (QT: 491 ± 80 ms, QTc: 353 ± 80 ms, T_{peak}-T_{end}: 49.8 ± 12 ms, mean ± SD) compared to the active period (QT: 332 ± 25 ms, QTc: 283 ± 29 ms, T_{peak}-T_{end}: 32.5 ± 3.3 ms). These ECG differences were also observed in the bear that experienced the VT episodes. We observed no spontaneous VT during the active period.

Conclusions: Polymorphic VT can be initiated by a PVC with an R-on-T phenomenon in brown bears, as in humans. Brown bears experience seasonal changes in electrophysiology, which may play a role in arrhythmogenesis. Further research is needed on the incidence of these arrhyth- mias, and understanding the mechanisms behind how the arrhythmias self-terminate may in- spire biomimetic approaches to human research.

P2_9: Generating Integration-Free Porcine Induced Pluripotent Stem Cells by Episomal Reprogramming

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The differentiation of iPSCs into cardiomyocytes provides a valuable *in vitro* platform for preclinical drug development and assessment of pre-arrhythmic effects, potentially reducing reliance on experimental animals in cardiac safety pharmacology. However, limited access to native human cardiac tissue poses challenges for validating human iPSC-derived cardiac models. To address this, we aim to generate iPSC-derived cardiomyocytes from pigs, one of the major cardiovascular animal models, with available *in vivo* data for comparison and model validation. Despite advancements in mouse and human iPSC generation, derivation of clinically relevant integration-free iPSCs in other species remains challenging. Here, we present our initial findings on the use of all-in-one episomal vectors containing human reprogramming factors to establish integration-free porcine iPSCs. Episomal reprogramming is a strategy to circumvent the genomic integration associated with the original viral-based reprogramming methods. Porcine fibroblasts isolated from a neonatal piglet were nucleofected with episomal vectors and plated for colony formation. Putative colonies were manually picked for clonal expansion and characterization. During subculture, the majority of clonal lines displayed a heterogeneous morphology indicative of differentiation and partial reprogramming. Three clonal lines successfully transitioned from culture on mitotically inactivated feeders to matrigel. Out of these, one was integration-free and exhibited pluripotency marker expression confirmed by both immunofluorescence staining and qPCR analysis. Ongoing efforts are focused on fully characterizing the pluripotency of the integration-free clonal line assessing its ability to differentiate into the three germ layers before directing differentiation towards the cardiac lineage to test for species-specific electrophysiological responses to pharmaceuticals.

P3_1: Transmission Electron Microscopy of Mitochondria in Horse Atrial Fibrillation

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Introduction: Atrial fibrillation (AF) is a common arrhythmia in both horses and humans, associated with structural and metabolic atrial remodeling. Mitochondrial dysfunction has been linked to AF progression in humans. This study aimed to longitudinally track the effects of AF on horse atrial mitochondrial ultrastructure using transmission electron microscopy (TEM).

Methods: Nine retired Standardbred horses were assigned to placebo (n=6) or sham (n=3) groups. Atrial fibrillation was induced by tachypacing and sustained for four months in the placebo group. Transvenous biopsies were collected from the right atrium at baseline, 2 weeks, 1, 2 and 3 months and processed for TEM imaging. A deep learning image segmentation model, MitoHorse, was trained and validated specifically for detecting mitochondria in horse cardiomyocytes. The generic MitoNet model, originally trained on mitochondria from other species, was used for comparison. Quantitative analysis of selected morphological parameters, including mitochondrial volume density, profiles per cell area, size, aspect ratio and circularity was performed.

Results: The MitoHorse model achieved an average Intersection over Union (IoU) of 0.92, outperforming the original MitoNet model (IoU = 0.48). Using the MitoHorse model with manual corrections, we observed no changes in the selected morphological parameters between groups or across time points ($p > 0.05$ for all comparisons).

Conclusion: Four months of chronic AF did not induce significant alterations in the ultrastructural characteristics of atrial mitochondria in horses. Future studies will explore whether horses are uniquely protected against mitochondrial dysfunction caused by AF.

P3_2: The role of KV1.5 in proliferation and collagen secretion upon NLRP3 inflammasome activation in cultured cardiac fibroblasts.

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide. Enhanced activity of the NACHT, LRR, and PYD domain containing protein 3 (NLRP3) inflammasome has been observed in several cardiac cell types including cardiomyocytes and cardiac fibroblast (CFB) in both AF patients and AF animal models. In cardiomyocytes, this enhanced NLRP3 inflammasome activity has been shown to be accompanied by an increase in the ultrarapid delayed rectifier potassium current (I_{Kur}), which promotes arrhythmogenesis. Although the enhanced NLRP3 inflammasome activity is associated with fibrotic remodelling, the exact role of enhanced activity of the NLRP3 inflammasome in CFB and the role of I_{Kur} remains elusive.

Aim: To investigate proliferation, collagen secretion, and the role of I_{Kur} in CFB upon activation of the NLRP3 inflammasome.

Methods: CFB were isolated from the right atrial appendage of porcine hearts. Immunocytochemistry of CFB was performed using 4′,6-diamidino-2-phenylindole (DAPI, nucleus marker), vimentin (fibroblast marker), and α-smooth muscle actin (myofibroblast marker). CFB were treated with lipopolysaccharide (LPS) + nigericin to activate the NLRP3 inflammasome and diphenyl phosphineoxide-1 (DPO-1) to inhibit the I_{Kur}. Protein levels of the NLRP3, caspase-1, pro caspase-1, interleukin (IL)-1β, pro IL-1β, IL-18, and pro IL-18 were quantified by western blotting. Collagen secretion by CFB was detected using Sirius Red Total Collagen Detection Assay. Proliferation was assessed using Axion Bioscience Omni FL. I_{Kur} was assessed by patch clamping using an EPC9-amplifier and measuring currents before and after addition of DPO-1.

Results: The abundance of NLRP3, caspase-1, pro caspase-1, IL-1β, pro IL-1β, IL-18, and pro IL-18 in CFB showed no statistically significant difference upon treatment with LPS + nigericin and/or DPO-1. Treatment with LPS + nigericin revealed a slight numerical increase in collagen secretion. Treatment with LPS + nigericin indicated a decrease in proliferation, while treatment with DPO-1 indicated an increase in proliferation, although without statistical significance in both instances. A numerical decrease in I_{Kur} was found by patch clamping after the addition of DPO-1, however the differences in current were not found to be statistically significant.

Conclusion: The study revealed the presence of the NLRP3 inflammasome components and I_{Kur} in porcine CFB. Activation of the NLRP3 inflammasome as well as the inhibition of the I_{Kur} did not result in changes in protein levels of NLRP3, caspase-1, pro caspase-1, IL-1β, pro IL-1β, IL-18, and pro IL-18 as well as the collagen secretion. However, LPS + nigericin and DPO-1 treatment did seem to affect the cell proliferation, which warrants further investigations.

P3_3: Proteomic Profiling of remodeling in hearts of an animal model of Cardiometabolic Syndrome

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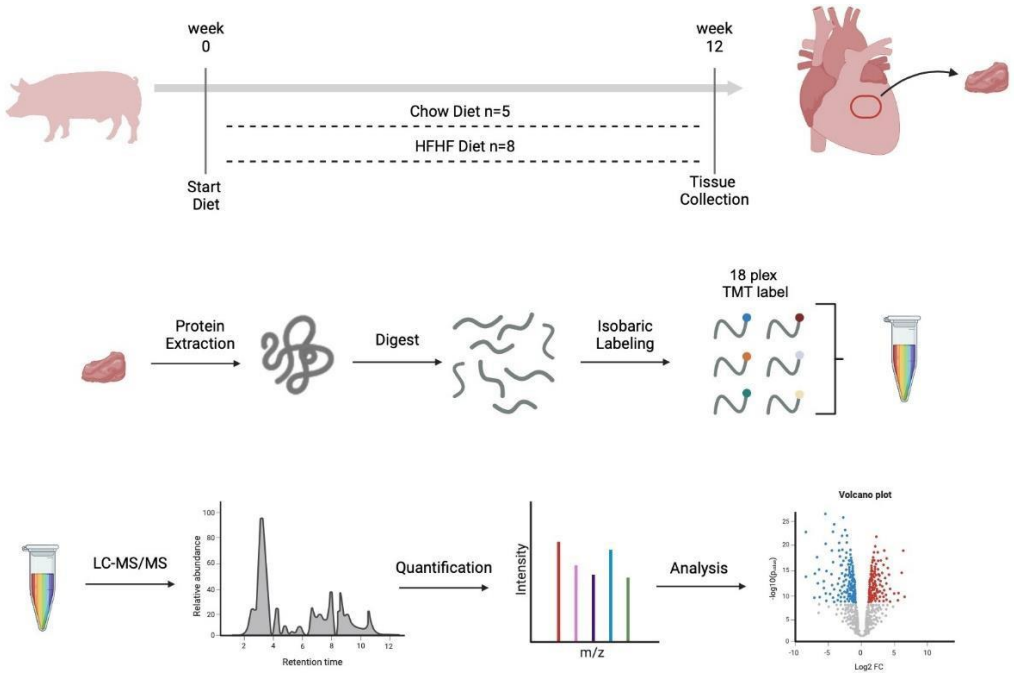
The wild pigs of Ossabaw Island, USA, exhibit a remarkable propensity for obesity and develop metabolic syndrome (MetS) when subjected to prolonged high-energy diets: closely resembling the state observed in metabolically unhealthy obese humans. Within nine weeks on a high-fat, high-fructose (HFHF) diet, Ossabaw pigs nearly double their body fat percentage and exhibit symptoms of MetS including a cluster of risk factors for cardiovascular disease. Consequently, the Ossabaw pig presents as an animal model for studying obesity and MetS associated diseases.
Herein, we set out to investigate the proteomic changes in hearts associated with cardiometabolic syndrome. To this end, we investigated cardiac protein remodeling consequent to diet-induced obesity in the Ossabow pig model. Thirteen Ossabaw pigs were fed either a HFHF diet or a chow diet for 12 weeks. By the end of the 12-week period, the pigs on the HFHF diet exhibited a significant increase in body weight compared to the pigs on the chow diet. Heart tissue samples were collected from the ventricular wall and analyzed by quantitative proteomics using a TMT labeling approach. The approach led to measurement of more than 6,000 proteins. The protein remodeling in the hearts of the obese animals is evaluated for regulation of proteins involved in insulin signaling pathway, as well as protein remodeling observed in hearts of obese humans, in addition to global remodeling analyses. By analyzing the proteomic differences between pigs fed on the different diets, we outline the cardiac protein remodeling in the Ossabaw pig model for cardiometabolic syndrome.



P3_4: Characterization of adipose tissue in human atrial appendages

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Background:

Cardiac adipose tissue plays a crucial role in maintaining healthy cardiac function. It communicates with the myocardium through paracrine signaling and provides free fatty acids for beta-oxidation. However, an increase in cardiac adipose tissue has been associated with a higher risk of arrhythmias. This risk is linked to the release of adipokines, which signal fibroblasts to increase fibrosis and alter ion channel profiles in cardiomyocytes. These changes can lead to prolonged action potentials and reentry circuits. Additionally, intramyocardial adipocytes can act as conduction blocks, slowing down conduction velocity and further increasing the risk of arrhythmias. In this study, we examine the adipose tissue of human atrial appendages in terms of fibrosis, adipose tissue volume and adipocyte size.

Methods:

The left and right atrial appendage was collected from 40 patients undergoing cardiac surgery at Rigshospitalet, Copenhagen (Denmark). Formalin fixed and paraffin embedded tissue was sectioned at 4µm and stained with Picro Sirius Red. The sections were imaged using the AxioScanZ1 and subjected to automated intelligent image segmentation using the QuPath software in order to quantify fibrosis, adipose tissue and adipocyte size.

Results:

We found that the content of fibrosis was significantly higher in the right atrial appendage (54.4 %) compared to the left (35.1 %) (p= >0.001)). In opposition, we found that the left appendage had a significantly higher amount of epicardial fat (35.0 %) compared to the right (11.2 %) (p= >0.001). Infiltration of intramyocardial fat was also found to be higher in the left appendage (0.4%) compared to the right (0.15 %) (p=0.02). Furthermore, we also observed that the intramyocardial adipocytes are smaller compared to epicardial adipocytes.

Conclusion:

We show structural differences between left and right human atrial appendages in terms of fibrosis, epicardial adipose tissue and infiltrating adipocytes, which potentially could contribute to our understanding of arrhythmia pathogenesis.

Future work:

Future work will leverage the unique opportunity of having both left and right atrial appendages for comparative analysis in our patient cohort. First, we will investigate if the structural characteristics have a predictive value for post-operative atrial fibrillation development and then further mechanistical investigate the role of fibrofatty infiltration for arrhythmia development.

P3_5: Acute Effects of Neural Specific Pulsed Field Ablation at Epicardial Ganglionated Plexi Sites on Atrial Refractoriness and Atrial Fibrillation: Histological and Immunohistochemical Analysis in a Porcine Model.

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Background: Pulse field ablation (PFA) is a non-thermal energy method for treating atrial fibrillation (AF). Endocardial delivery of PFA has been shown to have very limited to no impact on the ganglionated plexi (GP), which are located within the epicardial fat pads.

Objectives: To investigate the acute effect of epicardial PFA delivery on atrial electro-physiology and elucidate structural and molecular changes using histological analysis (HE-staining) and immunohistochemical staining with S100 antibody.

Methods: Ten pigs (three groups: 1. PFA (n=6), 2. sham-operated (n=2) and 3. control (n=2)). Thoracotomy was performed and six epicardial GP-anatomical regions were identified. Saline- irrigated PFA (1000V, 100µs, 12-QRS electrocardiogram [ECG]-synchronised pulse sequences) was applied to each GP-site. Local atrial electrogram (EGM) amplitude, atrial effective refractory period (aERP) and AF-inducibility/duration were assessed. Histology and immunohistochemistry were performed on treatment-adjacent structures.

Results: In six animals, 12-PFA pulses were successfully delivered to each of the six GP-target sites. Despite small transient injury currents, no difference in local atrial EGM amplitude was observed after PFA application (1.83±0.41mV vs. 1.92±0.53mV, p=0.72). In group 1, aERP measured in the high right atrium (RA) increased from 119±44 ms at baseline to 197±34 ms after PFA delivery (66% increase; 95% confidence interval, 20.5–153.5; p=0.02). Similarly, aERP in the low RA increased from 133±20 ms at baseline to 189±56 ms following treatment at all sites (42% increase; 95% confidence interval, 10.16–101.5; p=0.0256). AF-inducibility, decreased from 100% to 33%, with inducible AF-duration decreasing 4.7min at baseline to 1.3min post-PFA (mean difference -3.2±1.8 min, p=0.007). Macroscopic examination showed intact epicardial fat pads without signs of damage post-PFA. GPs within these fat pads were successfully identified using HE- staining, with preservation of adjacent atrial myocardium, extracellular matrix and fat tissue. Immunohistochemical analysis revealed significantly lower intensity of S100-protein expression within GP cytoplasm and membrane.

Conclusion: Epicardial PFA targeting GP-rich regions is feasible, prolonging aERP, reducing AF- inducibility and duration. Macroscopic, histological and immunohistochemical analyses revealed tissue and GP preservation, with decreased S100 protein intensity.

P3_6: Workflow for implementation of 3D-electroanatomical mapping and cardiac magnetic resonance imaging of atrial ablation lesions in pigs.

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Background: Atrial fibrillation (AF) ablation is the cornerstone of AF treatment in humans. Pigs are often used in preclinical and translational research due to the many similarities between the cardiac anatomy of pigs and humans. 3D-electroanatomical mapping (EAM) and cardiac magnetic resonance (CMR) imaging offers a comprehensive non-invasive approach to characterize and longitudinally track atrial substrates, including ablation lesions.

Methods and results: In 14 anesthetized female Danish landrace pigs we demonstrate that EAM by a high-density mapping catheter of the right and left atrium through transseptal puncture was reproducible and safe. Isolation of the pulmonary common trunk in the left atrium and an intercaval line could be placed reproducible in all pigs. Pigs were transferred from the preclinical facilities to the magnetic resonance scanner in the hospital, where QRS-triggering and breath-holding techniques were used to obtain clear and detailed images of the atria of all pigs by late gadolinium enhancement sequences. Images could be subsequently aligned with the EAM, facilitating comprehensive visualisation and analysis of fibrotic tissue.

Conclusion: Herein we describe a workflow of implementing EAM and CMR in a pig model to allow a multimodal and longitudinal assessment of atrial substrates and ablation lesions in the future.

P3_7: Towards structure-based improved treatments for atrial fibrillation

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Atrial fibrillation (AF) is the cause of one-third of all hospitalized heart rhythm disturbances and is associated with an increased risk of stroke and heart failure. The potential drug targeting of small-conductance calcium-activated potassium channels (SK-channels) situated in the cardiomyocytes' plasma membrane offers a promising avenue for treating atrial fibrillation. This approach aims to mitigate the severe side effects associated with existing treatments. Activation of calcium involves the interaction between the C-termini of SK channels and calmodulin (CaM), directly impacting the heart's action potential. This project seeks to uncover novel perspectives on the core structure, function, and pathophysiology of SK-channels, offering a foundation for refining compounds to intervene against atrial fibrillation.

Aims:

- Determine the first structure of human SK-channels 1-3 expressed in HEK293 cells using cryo-EM to shed light on their molecular function
- Obtain a high-resolution structure of SK-channel in complex with AF-modulator(s)
- Commence structure-based drug-optimization efforts including preclinical validation in collaboration with pioneers within AF therapies, Acesion Pharma.

This integration of structural biology and drug-discovery systems including electrophysiology will attain a new level of understanding of SK-channels in the pathology and treatment of AF. We have successfully obtained purified human SK1 from yeast cells and are transitioning to human cells.

P3_8: Three-dimensional characterization of cardiac innervation in horses with atrial fibrillation

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Background incl. aims
Activation of the autonomic nervous system (ANS) and local sympathetic hyperinnervation plays a pivotal, yet poorly understood role in the initiation and maintenance of atrial fibrillation (AF). Changes in the intricate 3-dimensional (3D) network of nerves are difficult to characterize using traditional histological methods. To gain insights into the role of innervation in cardiac arrhythmia, non-destructive imaging techniques capable of visualizing large tissue samples from large animal models of AF are crucial. Here we aimed to investigate the feasibility of 3D light sheet fluorescence microscopy (LSFM) in equine atrial tissue and provide a detailed 3D characterization of the sympathetic nervous system in the epicardium, myocardium and endocardium, while exploring innervation changes in a horse model of experimentally induced chronic AF.

Methods
Biopsies from the left posterior atrial wall were harvested from horses after 4-months of induced AF (n = 9), from horses with naturally occurring AF (n = 3) and healthy control horses (n = 3). A neuronal marker (Tyrosine hydroxylase) was stained to determine the local density of sympathetic nerves for computational image analysis. Whole-mount immunohistochemistry and clearing was optimized for equine heart samples by testing different depigmentation, permeabilization and imaging protocols.

Results
We demonstrate a protocol for 3D imaging of nerves in large equine atrial samples. Optimized sample preparation with stepwise chemical and enzymatic extracellular matrix loosening and digestion enabled uniform sample labelling with antibodies against a neuronal marker. Customized autofluorescence bleaching, sample clearing and imaging parameters made high resolution imaging of cardiac innervation feasible throughout the large tissue sample. Computational analysis of atrial innervation permitted quantitative analysis in the three groups to demonstrate spatial changes occurring in AF.

Conclusion
3D LSFM in large animal models can deepen our understanding of the autonomous nervous system´s role in AF development through enabling single-cell resolution imaging in dense cardiac biopsies. We show the applicability of the method to characterize innervation changes in an equine model of AF.

Graphic

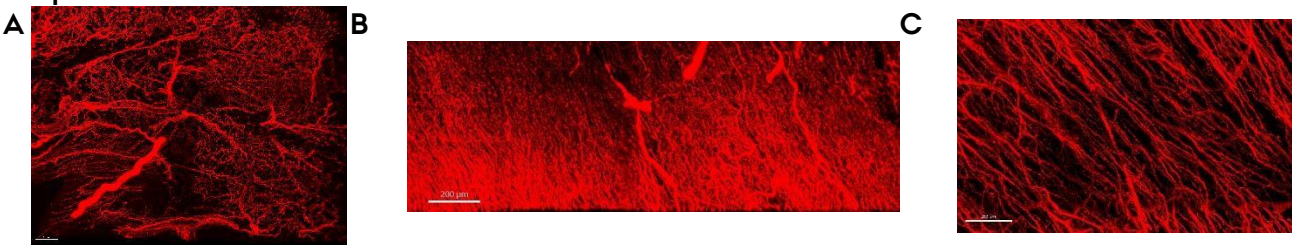

Fig. 1: Bird´s eye view of different layers of equine left atrial wall after 4-months of induced AF stained with sympathetic marker (Tyrosine hydroxylase, red). A) Epicardial layer with fat pad. B) Densely innervated myocardial layer. C) Endocardial layer.

P3_9: Proteomic remodeling of the heart following transplantation: the signature of cardiac allograft vasculopathy

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The major detriment to long-term survival with a transplanted heart is cardiac allograft vasculopathy (CAV). The molecular pathophysiology of CAV is poorly understood. Once CAV is diagnosed treatment is ineffective, but early (preangiographic) detection would enable a more timely treatment strategy. The aim of the study is to investigate the early changes in the heart proteome following a transplantation and to identify differences associated with CAV development.

As part of routine clinical care following heart transplantation, the graft is monitored by collecting endomyocardial biopsies at defined time points. These are currently evaluated by histology, but some formalin fixation and paraffin embedding (FFPE) tissue remains enabling research into new diagnostic strategies. We have established a state-of-the-art workflow to perform deep quantitative mass spectrometry proteomics measurements from cardiac FFPE samples on a timsTOF pro instrument (diaPASEF). To appropriately analyze proteomics data with repeated measurements taken from the same subject we implemented a regression strategy with an adequate within-subject correlation structure.

We identified more than 5000 unique proteins across 200 endomyocardial biopsies, corresponding to 40 patients and 5 time points per patient. We observed a substantial change in the proteome over time as the graft adapts to its new environment in the transplant recipient. Some proteins are associated with the formation of CAV.

This study adds to the understanding of the early adaptation of a transplanted human heart and could potentially lead to identification of biomarkers for early CAV detection.

P4_1: Machine Learning Prediction of White Matter Lesions to Infer Cerebral Comorbidity Risk Factors in AF and ACM Patients

Astrid Filt, Pia R. Lundgaard, Morten Steen Salling Olesen

Presenting Author

Astrid Filt

White Matter Lesions (WML's) are changes in the cerebral tissue caused by small cerebral infarctions. Such infarctions may be caused by atrial fibrillation (AF) and/or atrial cardiomyopathy (ACM). Patients with AF have an increased risk of stroke, heart failure (HF), dementia and death. In fact, one in three ischemic stroke patients have been found to have either symptomatic or asymptomatic AF. Many of these strokes could potentially have been prevented with oral anticoagulant (OAC) therapy, which has been shown to reduce stroke by 64%. Additionally, post-stroke studies have shown that up to 75% of AF episodes are asymptomatic. These results demonstrate the limited eRicacy of trying to detect AF through symptoms and using it for stroke prediction. These findings suggest that a significant reduction in stroke rates can be achieved by improving the ability to predict cardioembolic stroke. Rooted in our hypothesis - "Atrial cardiomyopathy is an important risk factor for AF and an independent predictor of white matter lesion formation and secondary embolic stroke." - this project utilizes machine learning models to firstly map and later subgroup patient MRI scans based on the presence of WML's and their history of AF and/or ACM. After examining the relationship between WML's and the presence of AF and/or ACM in patients, we dive into their genetics in an eRort to locate genetic markers that predispose for WML's in the hopes that such markers can be utilized in the clinic to take preventable steps, thus bringing down the number of AF related strokes. The machine learning models will be trained on a dataset of 1000 manually annotated cerebral MRI's. Once the performance and model architecture is satisfactory, the model will be used to annotate 40,000 cerebral MRI's from UK biobank.

P4_2: Machine Learning And Survival Analysis: A Study on Cardiovascu- lar Event Risk in Diabetes Patients from the English Longitudinal Study of Ageing

Benjamin Lebiecka-Johansen[1], Adam Hulman[234]

Survival analysis is well established in clinical predictions and epidemiology, with the Cox Proportional Hazard model underpinning several clinically implemented cardiovascular risk models, including the Framingham Risk Score, QRISK3, and SCORE2 risk predictions. However, as more diverse clinical data becomes available, including non-tabulated data such as electrocardiography, retinal images, and continuous glucose measurementsm regression models face limitations. Machine learning, with its wide array of models and algorithms, can handle multi-dimensional data, offering a potential solution.

In this study, we compare the performance of regression models with two machine learning models, Random Survival Forest and DeepHit, in predicting cardiovascular event risk. We examine how these models behave when adding more variables, such as physical measures (grip strength, leg rises, waist to hip ratio, blood pressure), blood samples (triglycerides, cholesterol, fasting lipids), etc. Model performance is assessed using C-statistics for discrimination and Brier score for calibration. This study is currently ongoing, and as such, results are not yet available.

Limitations: The study is based on a cohort of 7828 individuals older than 50 years of age at the study start. The individuals has volunteered and may not be representative of the background population. Furthermore, the data containing biomarkers has upwards of 30% missing data. This potentially impact the accuracy and generalizability of our findings.

Our findings provide insights into the conditions under which regression models are preferred over machine learning models and vice versa.
An outcome of this study is a clinical prediction pipeline made available according to the FAIR (findable, accessible, interoperable, and reusable) principles, ensuring the reproducibility of our research.

Future work includes expanding the study cohort using the Danish national registries, which will allow us to investigate the use of ECG data as input to machine- and deep learning models for predicting cardiovascular disease risk in diabetes patients. This could potentially enhance the predictive power of the models and provide more comprehensive risk assessments for patients.

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P4_3: Non-linear genetic regulation of the blood plasma proteome

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Abstract:

Although thousands of genetic variants are linked to human traits and diseases, the underlying mechanisms influencing these traits remain largely unexplored. One important aspect is to understand how proteins are regulated by the genome by identifying protein quantitative trait loci (pQTLs). Beyond this, there is a need to understand the role of complex genetics effects that regulate plasma proteins and protein biomarkers. Therefore, we developed EIR-auto-GP, a novel deep learning-based approach, to identify complex genetic effects regulating blood plasma proteins. Applying this method to the UK Biobank proteomics cohort of 52,700 individuals, we identified 171 proteins that were regulated by non-linear effects including non- linear covariates, genetic dominance and epistasis. We uncovered a novel epistatic interaction between the ABO and FUT3 loci, and demonstrated dominance effects of the ABO locus on plasma levels of pathogen recognition receptors CD209 and CLEC4M. Furthermore, we replicated these findings and the methodology across Olink and mass spectrometry-based cohorts and found that large sample sizes are needed to discover complex genetic effects. Our approach presents the first systematic, large-scale attempt to identify complex effects of plasma protein levels and can be applied to study other tissue or molecular QTLs.

P4_4: Illuminating the Potentials of Photoplethysmography in Patients with Atrial Fibrillation Using Explainable Artificial Intelligence

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Photoplethysmography (PPG) uses light to detect volumetric changes in the peripheral vasculature. It is a non-invasive and simple technology that provides truly continuous, long-term recordings and presents a compelling opportunity for new and expanded applications. However, PPG is less well characterised than electrocardiography (ECG), and it is unknown how risk factors, hemodynamic changes relating to atrial fibrillation (AF), and ablation therapy are reflected in the PPG. Furthermore, PPG interpretation is not as common compared to ECG interpretation, and automated analysis with artificial intelligence (AI) is likely to become the de facto standard. The purpose of this project is to use explainable artificial intelligence (xAI) to discover the unexplored clinical potentials of PPG as an assessment tool for patients with AF.

I will develop and apply convolutional neural networks (CNN) for detecting hemodynamical patterns based on characteristics from three independent cohorts (>6500 patients). I will apply CNNs on PPG signals to (1) detect and investigate the impact of AF risk factors including sex, age, diabetes, hypertension, and heart failure on PPG signals, (2) explore how AF-related hemodynamic changes are reflected in PPG signals and (3) distinguish between a patient's hemodynamical pattern before and after they have received ablation therapy and characterise the differences. I will specifically develop and apply xAI methods for PPG analysis, to uncover the hidden decision-making of the CNN, and thereby the linkage between the PPG signal and the outcome.

This project will characterise the effect of prevalent risk factors, which will aid in determining to what degree PPG may be used as a fast and easy gatekeeper for further diagnostic work-up, potentially reducing the number of unnecessary tests for the benefit of patients and society. I will generate important knowledge of AF mechanisms and how hemodynamics are reflected in PPG signals, characterising unknown hemodynamical phenotypes of AF. Finally, this project will give mechanistic information on ablation as a treatment for AF and may eventually help inform personalized treatment decisions. Overall, this project will determine the role of AI in the analysis of PPG signals.

P4_5: Leveraging Plasma Proteomics for Accurate Abdominal Aortic Aneurysms Predictions

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Background
Abdominal aortic aneurysms (AAA) pose a life-threatening risk due to unexpected rupture of the aortic wall, but current methods often fail to identify asymptomatic cases. Simple blood tests for detection outside hospital settings are urgently needed.

Aim
We aimed to assess whether specific proteins may provide additional value beyond clinical variables in a screening-based cohort that includes more patients than previously published studies.

Methods
Mass spectrometry analysis was performed on human plasma from 486 patients with AAA and 194 control subjects from the Viborg Vascular (VIVA) screening trial. Logistic and linear regression models were used for prediction of AAAs and aortic diameter, respectively. Performance was measured using either the area under the curve (AUC) for the detection of AAAs or the root mean square error (RMSE) for aortic diameter prediction, using 10-fold cross-validation.

Results
The inclusion of six proteins improved the AUC for detecting AAAs from 0.80 (using only clinical variables) to 0.84. The aortic diameter could be predicted based solely on clinical variables with an RMSE of 0.23. No additional proteins could improve the prediction of the aortic diameter.

Conclusion
We demonstrated the promising use of plasma protein data, in addition to clinical variables, for early diagnosis and growth prediction of AAA. This approach might contribute to the development of future screening methods.

P4_6: A New Practical Approach Toward Self-Powered Leadless Pacemakers by Heart-Kinetic Energy Harvesting Technology

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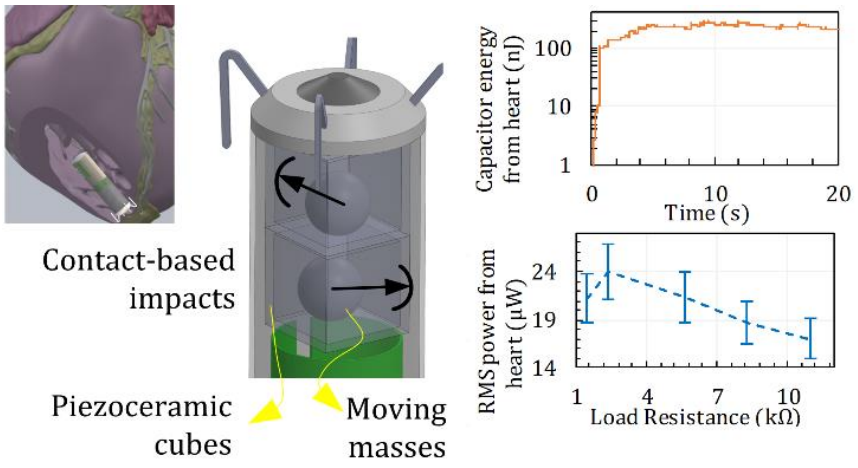
Leadless pacemakers rely on batteries for operation, and due to the surrounding tissue, they cannot be replaced in the long term. Our team has developed a method to generate energy from the heart's kinetic energy. We have demonstrated that this energy harvester can generate sufficient power for a leadless pacemaker.

We are working on the long-term use and modification of this system. Our team is focused on three aspects of the project: the electrical circuit for charging, the piezoelectric material for long-term use, and the kinetic analysis of the heart for optimizing the impact.

We have started a long-term test with an accelerated lifetime test using a heart simulation signal in the laboratory. The shaker created an acceleration of 80 m/s², much higher than the heart's 10 m/s². After one year of testing, SEM images and X-ray spectrography showed that the piezoelectric material remained intact, but the electrode material was degraded due to the impact. The electrode was made of silver, which is soft and weak against impacts. Since this material was not optimized for impact, we are working to find a more resistant electrode coating. We have found a new material layout: Titanium as the center shim, with pure Titanium Nitride (TiN) as the top and bottom electrodes. The electrodes are created by thin layer deposition on the Titanium substrate. A COMSOL transient model was created to precisely measure the impact of a moving ball, emphasizing the electrical distribution in the piezoelectric structure due to impact.

We are also developing a new sensor for measuring heart kinetic motion in animal trials. This sensor not only measures translational linear acceleration but also calculates rotational acceleration, which, based on our laboratory and animal test results, is an important factor. We placed this sensor inside a cylinder like the Micra leadless pacemaker and inserted it into the heart.



P5_1: Prognostic Impact of Iron Deficiency in New-onset Chronic Heart Failure: Danish Heart Failure Registry Insights.

Abdullahi Ahmed Mohamed MD, Daniel Mølager Christensen MD, PhD, Milan Mohammad, Christian Torp-Pedersen MD, DMSc, Lars Køber MD, DMSc, Emil Loldrup Fosbøl MD, PhD, Tor Biering-Sørensen MD, MSc, MPH, PhD, Morten Lock MD, PhD, Mariam Elmegaard Malik MD, Nina Nouhravesh MD, Charlotte Anderrson MD, PhD, Morten Schou MD, PhD, Gunnar Gislason MD, PhD,

Aims:
Iron deficiency (ID) is prevalent in chronic heart failure (HF) but lacks a consensus definition. This study evaluates the prevalence of ID and its association with all-cause and cardiovascular mortality, as well as first hospitalization for HF, using current European Society of Cardiology (ESC) guidelines, ferritin <100 ng/ml, TSAT <20%, and iron ≤13 µmol/L as criteria for ID.

Methods and Results:
Of 9477 new-onset chronic HF patients registered in the Danish Heart Failure Registry from April 2003 to December 2019, we observed ID prevalence rates ranging from 35.8% to 64.3% depending on the ID definition used. Regardless of anemia status, ID defined by TSAT <20% and serum iron ≤13 µmol/L was associated with all-cause mortality (non-anemic, HR: 1.57, 95% CI:1.30-1.89 and HR: 1.46, 95% CI: 1.24-1.73; anemic, HR: 1.22, 95% CI: 1.07-1.38 and HR: 1.26, 95% CI: 1.10-1.44, respectively) and cardiovascular mortality (non-anemic, HR: 1.93, 95% CI:1.49-1.2.49 and HR: 1.53, 95% CI: 1.22-1.91; anemic, HR: 1.29, 95% CI: 1.10-1.52 and HR: 1.30, 95% CI: 1.10-1.55, respectively), as well as increased risk of first hospitalization for HF (non-anemic, HR: 1.26, 95% CI:1.09-1.1.50 and HR: 1.27, 95% CI: 1.11-1.47; anemic, HR: 1.25, 95% CI: 1.08-1.44 and HR: 1.22, 95% CI: 1.05-1.44, respectively)). ID defined by ESC guidelines was associated with all-cause and cardiovascular mortality only in non-anemic patients (HR: 1.41, 95% CI:1.18-1.1.69 and HR: 1.47, 95% CI: 1.15-1.87). Furthermore, the ESC guideline definition was association with increased risk of first hospitalization for HF, regardless of anemia status (non-anemic, HR: 1.26, 95% CI:1.08-1.1.47; anemic, HR: 1.34, 95% CI: 1.17-1.53).

Conclusions:
ID, when defined by TSAT <20% or serum iron ≤13 µmol/L, is associated with increased risk of all-cause and cardiovascular mortality, as well as first hospitalization for HF in patients with new-onset chronic heart failure, regardless of anemia status. Conversely, ID defines as ESC guidelines is associated with all-cause and cardiovascular mortality only in non-anemic patients.

P5_2: Elevated remnant cholesterol and atherosclerotic cardiovascular disease in diabetes: a population-based prospective cohort study

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Aims: Elevated remnant cholesterol is observationally and causally associated with increased risk of atherosclerotic cardiovascular disease (ASCVD) in the general population. This association is not well studied in individuals with diabetes, who are often included in clinical trials of remnant cholesterol lowering therapy. We tested the hypothesis that elevated remnant cholesterol is associated with increased risk of ASCVD in individuals with diabetes. We also explored the fraction of excess risk conferred by diabetes which can be explained by elevated remnant cholesterol.

Methods: We included 4,569 white Danish individuals with diabetes (59% statin users) nested within the Copenhagen General Population Study (2003-2015). The ASCVDs peripheral artery disease, myocardial infarction, ischemic stroke were extracted from national Danish health registries without losses to follow-up. Remnant cholesterol was calculated from a standard lipid- profile.

Results: During up to 15 years of follow-up, 236 individuals were diagnosed with peripheral artery disease, 234 with myocardial infarction, 226 with ischemic stroke, and 498 with any ASCVD. Multivariable adjusted hazard ratio (95% confidence interval) per doubling of remnant was 1.6 (1.1, 2.3; P=0.01) for peripheral artery disease, 1.8 (1.2, 2.5; P=0.002) for myocardial infarction, 1.5 (1.0, 2.1; P=0.04) for ischemic stroke, and 1.6 (1.2, 2.0; P=0.0003) for any ASCVD. Excess risk conferred by diabetes was 2.5-fold for peripheral artery disease, 1.6-fold for myocardial infarction, 1.4-fold for ischemic stroke, and 1.6-fold for any ASCVD. Excess risk explained by elevated remnant cholesterol and low-grade inflammation was 14% and 8% for peripheral artery disease, 26% and 16% for myocardial infarction, 34% and 34% for ischemic stroke, and 24% and 18% for any ASCVD, respectively. LDL cholesterol did not explain excess risk, as it was not higher in individuals with diabetes.

Conclusions: Elevated remnant cholesterol was associated with increased risk of ASCVD in individuals with diabetes. Remnant cholesterol and low-grade inflammation explained substantial excess risk of ASCVD conferred by diabetes. Whether remnant cholesterol should be used as a treatment target remains to be determined in randomized controlled trials.

P5_3: Patient characteristics and mortality trends among octogenarians with heart failure over two decades - a Danish nationwide study

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Background

Mortality rates among octogenarians with heart failure (HF) have changed little despite significant advancements in care over recent decades. Investigating the factors contributing to this trend may offer valuable insights for devising targeted management strategies for the oldest HF patients.

Purpose

We aimed to examine different patient characteristics associated with mortality among octogenarians with HF over the past 20 years.

Methods

From nationwide Danish healthcare registries, we included all patients with new-onset HF between 2001-2020 who were ≥ 80 years old at the time of diagnosis. The patients were categorized into 5-year calendar periods depending on the time of their diagnosis, the first period being between 2001-2005 and the last 2016-2020. The Kaplan-Meier estimator was used to assess the mortality risk. Mortality rates were examined with multivariable Cox regression models, comparing the first (reference) and the last calendar period and adjusting for predefined covariables.

Results

We included 47,754 octogenarian patients with new-onset HF, of whom 13,293 (51%) were diagnosed between 2001-2005 and 12,813 (49%) between 2016-2020. The distribution of males and females changed between the two calendar periods, with 5696 (43%) males in the first period and 6631 (52%) in the last period. The 3-year absolute mortality risk decreased by 8% [95% CI, 7.9-8.0] from 2001-2005 (64% [95% CI, 63.9-64.7]) to 2016-2020 (56% [95% CI, 55.3-56.7]) (p-value <0.001) (Figure 1). After adjustment for covariables, patients diagnosed in the last period exhibited a mortality hazard ratio (HR) of 0.94 [CI 95% 0.90- 0.99] (p-value 0.02) compared to those diagnosed in the first period. An interaction was observed between the first and the last calendar periods among patients with a history of myocardial infarction and those aged <85 years. The survival rates improved over time for patients with a history of myocardial infarction (HR 0.80 [95% CI, 0.72-0.90], p-value for interaction<0.001), as well as for those aged between 80 and 85 years (HR 0.86 [95% CI, 0.80-0.91], p-value for interaction<0.001) (Figure 2).

Conclusion

Over two decades the mortality risk among octogenarians with HF has improved slightly, however it remains high. Patients with a history of myocardial infarction and those aged 80 to 85 years emerge as subgroups experiencing the most improved survival rates over time.

P5_4: Temporal Trends in Medication Usage Before and During Pregnancy 1997-2023: A Danish Nationwide Study

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Background
Maternal medication use during pregnancy requires careful consideration of both maternal and fetal health. Historically, pregnant women have been excluded from drug trials, limiting the understanding of medication safety and efficacy in this population. This study aims to provide a comprehensive analysis of medication use trends among pregnant women in Denmark from 1997 to 2023, addressing detailed drug classifications and maternal demographic factors.

Methods
We conducted a nationwide cohort study, identifying pregnancies resulting in live births from September 1, 1997, to April 30, 2023, using Danish national registries. Medication use was defined by prescription redemption during the nine months before conception and throughout pregnancy. Trends were analyzed using Poisson regression for the 20 most prescribed medications at the ATC level 5. We examined the influence of maternal age, education, employment status, and urbanization on medication use patterns.

Results
Among 1,550,038 pregnancies, 58% of women redeemed at least one prescription during pregnancy, increasing from 29% in 1997 to 64% in 2023. Higher medication use was associated with older age, higher education, employment, and urban residence. Notable trends included a significant increase in the use of ondansetron and sertraline, and a shift from sulfamethizole to pivmecillinam for urinary tract infections. Antidepressant use showed a decline in citalopram and a rise in sertraline. These trends reflect evolving clinical practices and updated guidelines.

Conclusion
The study highlights significant shifts in medication use during pregnancy over 26 years, driven by changing clinical guidelines and demographic factors. The increasing medication use underscores need for ongoing monitoring, updated clinical guidelines, and targeted interventions to address disparities in healthcare access. Future research should focus on the safety of commonly prescribed medications and the impact of socio-demographic factors on medication use patterns.

P5_5: Myocardial Infarction Risk Across Cancer Subtypes – A Nationwide Registry-Based Study - A Nationwide Registry-Based Study

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Background: Remarkable progress in cancer diagnosis and treatment have significantly improved patient outcomes, leading to increased survival rates. However cardiovascular events due to different types of cancer is challenging and not well studied.

Purpose: The aim of this study was to examine the risk of Myocardial infarction in patients with different types of cancer compared with age- and sex-matched population control subjects with benign skin cancer.

Methods: Patients with cancer between 2000-2016 were identified within the Danish National Patient Registry. Absolute and relative risks of cardiovascular events were derived from logistic regression. Estimates were standardized to the distributions of age, sex, selected comorbidities, and pharmacotherapies of all included subjects.

Results: A total of 526.995 patients were included in the final analysis of whom 372.970 had cancer with different subtypes and 154.025 were control subjects with benign skin cancer. Differences in the prevalences of selected comorbid conditions, including diabetes, hypertension, and other risk factors for coronary artery disease and antianginal medications were not significant (P>0.05 for all).
The one-year outcome of myocardial infarction was overall significant higher for all subtypes of cancer especially Lymphoma OR 1.66 [1.45-1.89], Bladder cancer OR 1.65 [1.39-1.95] and Gastrointestinal cancer OR 1.54 (1.40-1.69] but Hepatobiliary and pancreatic cancer, Breast cancer and Malignant melanoma had OR 0.95 [0.79-1.14], 0.92[0.79-1.07] and 0.76[0.60-0.96] were not statistically significant.

Conclusions: The one-year of myocardial infarction varied across cancer subtypes. Notably, lymphoma, bladder, and gastrointestinal cancers exhibited higher risks, whereas hepatobiliary and pancreatic, breast, and malignant melanoma cancers showed no statistically significant associations. The clinical significance of these differences must be viewed within the context of the significant competing risk of death in this cancer population.

P5_6: The Interplay betewen Drinking Patterns, Metabolic Signatures, and Cardiovascular Disease Incidence: the CARDIOSIP study protocol

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Background: Cardiovascular diseases are a significant public health challenge, with alcohol consumption being one of their major modifiable exposures but with a controversial role. One persistent challenge is the inadequate characterization of individual drinking patterns in scientific studies. Further, biomarkers of low-to-moderate alcohol exposure and the mechanisms underlying the molecular processes are lacking.
Objectives: In CARDIOSIP, we aim to study the association between drinking patterns, cardiometabolic health, and the incidence of coronary heart disease (CHD) and stroke. Further, we aim to identify the metabolic signature of a regular low-to-moderate drinking pattern and evaluate its prospective association with CHD risk.
Methods: Two well-phenotyped Danish prospective cohort studies will be used (DANHES and Inter99), and the results will be externally replicated in U.S. cohorts (Nurses' Health Study and Health Professional's Follow-up Study). We will apply advanced epidemiological methods and machine-learning techniques. Cardiometabolic health status will be defined with five well- established pathways of metabolic health (hyperglycaemia, inflammation, dyslipidaemia, blood pressure, body weight and composition) and long-term CHD and stroke risk. The relationship between drinking patterns and cardiometabolic health will be evaluated using linear regression models. Cox proportional regression models will be used to calculate hazard ratios and their 95% coeaicient interval for the risk of CHD and stroke in relation to alcohol drinking patterns (DANHES cohort, n=76,484). The associations between metabolomic profiles at baseline and the incidence of fatal and non-fatal CHD will be evaluated. Elastic net regressions with training-testing sets (70- 30%) will be performed, and Cox proportional hazard models will be conducted (Inter99 cohort, n=3,000).
Significance: This project integrates nutrition, epidemiology, and new-omics data in established Danish cohorts. It aims to provide clues into the pathogenic and preventive role of alcohol in cardiometabolic health, such as the potential diaerential impact of specific beverages and its associated drinking pattern. Additionally, it seeks to identify new biomarkers specific for low-to- modeate alcohol to provide evidence regarding the type, dose, and frequency of alcohol exposure, and therefore their impact on cardiovascular health outcomes.

P5_7: Lipoprotein(a) cardiovascular disease risk not captured by LDL cholesterol and apolipoprotein B

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Background and aim: Elevated lipoprotein(a) affects 1 in 5 individuals and is considered a causal risk factor for atherosclerotic cardiovascular disease (ASCVD) and aortic valve stenosis (AVS). However, lipoprotein(a) measurements are not widely implemented in clinical practice. Low-density lipoprotein (LDL) cholesterol and apolipoprotein B (ApoB) measurements include contributions from lipoprotein(a), yet whether these measurements can replace measurements of lipoprotein(a) in assessing cardiovascular disease risk from lipoprotein(a) is unknown. Therefore, we tested the hypotheses that lipoprotein(a) risk of ASCVD and AVS is captured by LDL cholesterol or ApoB.

Methods: We included 70,189 individuals from the Copenhagen General Population Study, a contemporary prospective cohort study. Mediated risk by LDL cholesterol and ApoB in the association between lipoprotein(a) and risk of ASCVD and AVS was examined using four-way decomposition analyses.

Results: Per 50 mg/dL higher lipoprotein(a), multivariable adjusted hazard ratios were 1.22 (95% CI: 1.18-1.26) for ASCVD and 1.36 (1.28-1.46) for AVS. In non-statin users, LDL cholesterol mediated 19% (15%-24%) of the risk of ASCVD from lipoprotein(a) and 7.7% (1.5%-14%) of the risk of AVS from lipoprotein(a). Corresponding values for ApoB were 14.0% (11%-18%) and 6.6% (2.8%-10%). In statin users (n=9,570), LDL cholesterol did not mediate risk of ASCVD but mediated 8.0% (0.8%-15%) of the risk of AVS from lipoprotein(a). Results for ApoB were similar in statin users and non-users.

Conclusion: Lipoprotein(a) risk of ASCVD and AVS is not adequately captured by LDL cholesterol and ApoB. Therefore, lipoprotein(a) measurement is needed to capture the cardiovascular disease risk from this causal risk factor.

P5_8: Combination of cardioprotective glucose-lowering drugs and statins on all-cause mortality

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Background:
Sodium glucose cotransporter 2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1RA), collectively termed cardioprotective glucose lowering drugs (CPGLD), and statins are aimed at reducing complications to type 2 diabetes. We tested the hypothesis that combination of CPGLD and statins was associated with lower risk of all-cause mortality than using either drug alone.

Methods:
From December 2012 to December 2021 we identified individuals in Denmark with type 2 diabetes using: 1) CPGLD, 2) statins, 3) both, and 4) no treatment. We used three designs. First, in a Simple Cohort, we followed people by treatment status from January 2015 (197 507 individuals). Second in an Active Comparator Cohort we followed people initiating CPGLD or dipeptidyl peptidase-4 inhibitors (active comparator) as second-line treatment to metformin (75 285 individuals). Third, in a Time-varying Cohort we followed all people with type 2 diabetes and updated their treatment and covariate status annually (354 979 individuals). Depending on design, all-cause mortality was assessed using multivariable-adjusted cox with or without inverse probability for treatment weighting for confounder control.

Results:
Statin use was associated with lower risk of all-cause mortality compared to no treatment. Multivariable-adjusted hazard ratios: 0.76 (95% CI: 0.74-0.77), 0.77 (0.72-0.83), and 0.63 (0.62-0.64), and weight-adjusted
hazard ratios: 0.81 (0.79-0.83), 0.78 (0.72-0.84), 0.67 (0.66-0.69) for the Simple, Active Comparator, and Time-varying Cohorts. CPGLD use was associated with lower risk of all-cause mortality compared to no treatment. Multivariable-adjusted hazard ratios: 0.76 (0.70-0.82), 0.77 (0.70-0.85), 0.61 (0.58-0.64), and
weight-adjusted hazard ratios: 0.79 (0.69-0.91), 0.72 (0.63-0.82), 0.73 (0.65-0.81) for the Simple, Active Comparator, and Time-varying Cohorts. Combined statin and CPGLD use was associated with ~10-20% point reduction than either drug alone.

Conclusions:
In individuals with type 2 diabetes, using CPGLD and statins in combination was associated with a lower risk of all-cause mortality than using either drug alone.

P6_1: Effectiveness and Cost effectiveness of a Structured Follow up Program for Patients with Pulmonary Embolism: A clinical multicentre pre post study

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Aim (hypothesis): This PhD aim to investigate the effect of a structured follow-up care program (The ATTEND-PE model) for patients with pulmonary embolism (PE) compared with usual care. Furthermore, the cost-effectiveness and cost-utility of the follow-up care program will be investigated. Implementa-tion of the ATTEND-PE model is expected to improve patient-reported health related quality of life, disability, treatment satisfaction, mental health, and work productivity in patients with PE, compared with usual care. Furthermore, improves VTE-related mortality, recurrent PE, major bleeding, adherence with anticoagulant medicine and use of health care services. The ATTEND-PE model is expected to be cost-effective compared to usual care.

Background: Patients with PE experience impaired functional ability and reduced quality of life long after discharge from the hospital. However, there are no structured follow-up guideline or strategy in Denmark for these patients, and there is considerable variation in practice patterns of post-PE manage-ment. Furthermore, no studies have investigated the effectiveness of structured follow-up care models in patients with PE.

Methods: A pre-post study design is used, where the pre-implementation population will be compared to the post-implementation population on both patient-reported and clinical outcome measures, using appropriate statistical methodology. The cost-effectiveness of the ATTEND-PE model will be assessed using a cost-effectiveness analysis (CEA) and a cost-utility analysis (CUA).

Perspectives: This PhD project will be the first to examine the effectiveness of a structured follow-up program for patients with PE. It will serve to establish consensus on what an optimal follow-up strategy for post-PE care should include and inform future guideline development both nationally and interna-tionally. Additionally, this PhD will clarify whether the model is beneficial from a health economic per-spective. These results are crucial for informed resource allocation and improving the quality assurance of current healthcare services. An important step towards providing sustainable follow-up care post-PE that will improve the functional status and quality of life for patients who find everyday life impacted by PE.

P6_2: CHARACTERIZATION OF CARDIOVASCULAR AND RENAL DAMAGE, BOTH ACUTE AND LONG-TERM (SEQUENCES), IN A PRECLINICAL MODEL THAT MIMICS THE EFFECT OF THE CYTOKINE STORM ASSOCIATED WITH COVID-19

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The pandemic caused by the SARS-COV2 virus has forced the scientific community to accelerate tools to understand the molecular mechanisms related to the systemic inflammatory response syndrome, in infectious or non-infectious pathologies. It is also known that SARS-CoV-2 can cause extrapulmonary manifestations both during and after infection, including cardiovascular and renal complications.
The objective of this study is to characterize, in a preclinical model that mimics the effect of the cytokine storm associated with COVID-19, the damage to cardiovascular and renal function, both acute and long-term (sequelae).
Adult male Wistar rats of 3 months were divided into four experimental groups (n=8): a) Acute control: animals to which i.p. vehicle of LPS (saline serum) + vehicle of imiquimod (PBS) + vehicle of ATP (PBS) with follow-up of 3 days; b) Acute storm: animals to which
i.p. LPS (3 mg/kg) + imiquimod 0.1 mg/kg + ATP 5 mg/kg with follow-up of 3 days; c) Sequelae control: animals to which i.p. vehicle of LPS (saline serum) + vehicle of imiquimod (PBS) + vehicle of ATP (PBS) with follow-up of 3 weeks; b) Sequelae storm: animals to which i.p. LPS (1.5 mg/kg) + imiquimod 0.1 mg/kg + ATP 5 mg/kg with follow- up of 3 weeks. At the end of the experimental period, blood pressure and heart rate were analyzed by direct cannulation of the carotid artery, basal cardiac function by the Langendorff technique, aorta reactivity in an organ bath, and renal function with plasma creatinine levels. The indices of cardiac and renal masses were also evaluated.
The animals in which the cytokine storm was provoked had a decrease in blood pressure levels, which was not accompanied by changes in heart rate. In addition, these animals did not present any alteration in basal cardiac function, but they did show a slight hypo-contractile reactivity and increased vasodilation. There were no changes in plasma creatinine levels. Three weeks after provoking the storm, the animals recovered their blood pressure levels, showing no cardiac alterations or creatinine levels. However, three weeks after the cytokine storm, the animals showed hypercontractile reactivity in the aorta accompanied by significant endothelial dysfunction. These results suggest that the systemic inflammatory response syndrome causes long-term sequelae at the cardiovascular level that mainly affect the vascular system.

Keywords: Cytokine storm, vascular damage, COVID-19.
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P6_3: Capillary dysfunction is associated with impaired neurovascular coupling after ischemic stroke

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Introduction: Neurovascular coupling, as the local hyperemic response to neuronal activity, is impaired in peri-ischemic brain regions after stroke, with the mechanism being unknown. The mechanism is important for the control of brain blood flow, and its dysfunction after stroke, where reduced neurovascular coupling may contribute to futile reperfusion, i.e., poor neurological outcome despite successful recanalization. Understanding these mechanisms is therefore critical for the development of targeted therapy. We aimed to assess neurovascular responses and capillary perfusion in the peri-ischemic area before stroke and after ischemia reperfusion. It was hypothesized that impaired neurovascular coupling in the peri-ischemic area is associated with disrupted capillary microcirculation.

Methods: Six mice implanted with chronic cranial windows were trained for awake head-fixation prior to experiments. One-hour occlusion of the anterior middle cerebral artery branch was induced using single vessel photothrombosis. Cerebral perfusion and neurovascular coupling were assessed by optical coherence tomography and laser speckle contrast imaging. Capillaries and pericytes were studied in perfusion-fixed tissue by labelling lectin and platelet-derived growth factor receptor β.

Results: Arterial occlusion induced on average 11 spreading depressions over one hour associated with substantially reduced blood flow in the peri-ischemic cortex. Approximately half of the capillaries in the peri-ischemic area were no longer perfused after reperfusion (Figure), which was associated with a reduced diameter of capillaries surrounded by pericytes. The capillaries in the peri-ischemic cortex that remained perfused showed an increased prevalence of flow stalling. Blood flow velocity in these capillaries that remained perfused was similar at 3-hour follow- up but increased at the 24-hour follow-up compared with baseline. Whisker stimulation led to reduced neurovascular coupling responses in the sensory cortex corresponding to the peri- ischemic region 3 and 24 hours after reperfusion compared with baseline.

Conclusion. Arterial occlusion led to a reduceddiameter of pericyte-surrounded capillaries in the peri-ischemic cortex associated with microcirculatory failure. This reduced capillary capacity may, at least in part, underlie impaired neurovascular coupling in peri-ischemic brain regions after reperfusion. Control and correction of capillary capacity may reduce the neurological dysfunction after stroke as a target for therapy.

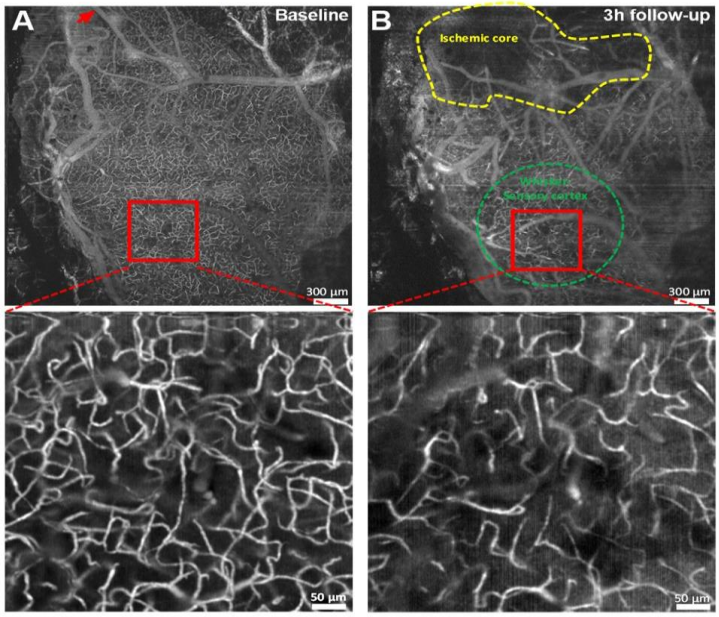

Figure. Representative optical coherence tomography (OCT) images of the affected hemisphere at baseline

Figure. Representative optical coherence tomography (OCT) images of the affected hemisphere at baseline
(A) and at the 3-hour follow-up (B). The red arrow indicates the location of photothrombosis (A). Yellow and green dotted areas indicate the ischemic core and the whisker sensory cortex, respectively (B). A reduced number of perfused capillaries was observed after ischemia-reperfusion

P6_4: Biological evaluation of KV7.1 ion channel modulators on rat penile tissue via myography.

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The voltage-gated Kv7 potassium channels are key regulators of smooth muscle contractility[1]. The corpus cavernosum of the penis contains smooth muscle that relaxes during an erection to allow blood to fill the sinusoids. This relaxation is nitric oxide-dependent and Kv7 channels might play a role in the relaxation of the corpus cavernosum smooth muscle. In erectile dysfunction, the blood flow to the penis and relaxation of the corpus cavernosum is impaired; however the precise mechanisms are not fully understood. Previously, we have shown the specific activation of the Kv7.1 channel could enhance sildenafil-induced relaxation of the corpus cavernosum [2]. This project aims to characterize the role of Kv7.1 channels in corpus canvernosum smooth muscle during an erection and whether these channels could be considered as a novel target to treat erectile dysfunction.

The known positive allosteric modulator of the KV7.1 channel R-L3, as well as new derivatives of R-L3, will be tested for their relaxing effects on the corpus cavernosum of rat penile tissue via myography.

[1] Stott *et al.,* Drug Discovery Today, **2014**, 19(4), 413-424

[2] Jepps *et al.*, British Journal of Pharmacology, **2016**, 173(9), 1478-1490

P6_5: The role of micro RNAs in the obese adipose vascular niche

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Background: Obesity is a prevailing health concern intimately linked to adipose tissue (AT) dysfunction, characterized by hypertrophied adipocytes (AC) that contribute to lipid overflow into vital organs. Adipose endothelial cells (adEC) are crucial for maintaining AT function and undergo changes in response to altered AC adipokine secretion during obesity, suggesting the importance of AC-adEC interaction for AT and metabolic health.

MicroRNAs (miRNA) are regulatory molecules that can be secreted and exchanged from the AT via extracellular vesicles (EVs), influencing gene expression in recipient cells. Obesity influences the secretion and expression of miRNAs in AT. However, the specific miRNAs that control crosstalk between AC and adEC and their significance in the obese vascular niche has not been addressed to date.

Preliminary research in my host laboratory has demonstrated abundant expression of miR-149 in mature AC upon high fat diet feeding, whereas miR-149 has been shown in another study to be repressed in obese adEC. This suggests a potential exchange of miR-149 between AC and adEC in metabolic disease.

Aim: This project aims to investigate the EV-mediated exchange of obesity- associated miRNAs between AC and adEC.

To achieve this, we will (1) integrate data from various omics datasets and single- cell databases to map obesity-related changes of miRNA:mRNA gene networks in adipose vasculature, impute miRNA activities, and identify potential miRNA candidates for further analysis, and (2) study the miRNA exchange in EV-mediated adEC-AC communication with genetically modified 'ExoRep' mice expressing CD9- eGFP^{+} EVs exclusively in AC and adEC.

P6_6: Preserved blood-brain barrier and neurovascular coupling in 5xFAD mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the leading cause of dementia where the pathological hallmark is the deposition of Amyloid-β (Aβ) peptide. Recent studies showed that dysfunction of brain blood vessels may play a causal role in AD. However, it is controversial whether parenchymal Aβ dysregulates the brain blood vessels in AD.

Here, we characterized the blood-brain barrier (BBB) and the neurovascular coupling in individual cerebral vessels in a mouse model of severe Aβ pathology (5xFAD mice *vs* wild-type littermates, WT; all 7–11-month-old) using *in vivo* two-photon microscopy, electrophysiology, and *ex vivo* immunohistochemistry.

We found that the BBB was preserved in the 5xFAD mice despite the presence of Aβ in the brain parenchyma: in the 5xFAD mice, both the paracellular transport of small molecules (Na fluorescein, 0.4 kDa) and the adsorption-mediated transcytosis of large molecules (albumin, 65 kDa) were as low as in the WT mice, indicating preserved barrier function of the blood vessels. Moreover, the 5xFAD mouse brains showed no signs of extravasation of fibrinogen (an endogenous marker of BBB integrity) and no alteration of the capillary pericyte numbers (critical for BBB function), confirming the preserved BBB in the 5xFAD mice. Finally, the neurovascular coupling was also preserved in the 5xFAD mice since vasodilation upon somatosensory stimulation was the same in 5xFAD and WT mice in penetrating arterioles, precapillary sphincters, and capillaries, i.e., the core vascular segments controlling the cerebral perfusion.

Thus, we showed that the parenchymal Aβ may be insufficient for dysregulating brain blood vessels in AD. Instead, factors such as the *vascular* Aβ aggregates or tau tangles (both are absent in 5xFAD mice) could cause cerebrovascular dysfunction in AD.

P6_7: A novel expansible aortic annuloplasty ring for aortic valve repair

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Introduction
50% of patients with aortic insufficiency (AI) undergoing aortic valve replacement will experience valve-related complications within ten years. This risk can be reduced to only 12% by repairing the aortic valve instead of replacing it. Although its promising perspective, aortic valve repair is conducted in only 14%, even though 80% of AI cases are deemed eligible for repair. A subvalvular annuloplasty ring is essential to avoid recurrent AI when performing aortic valve repair. Different annuloplasty rings exist, but none have proven superior in terms of material, shape or position and no guidelines on the subject of annuloplasty ring exist. Furthermore, none of the currently available annuloplasty rings have a heterogeneous construction. Finally, most are closed and cannot be used for isolated aortic valve repair.
To address this deficiency, our group has developed a physiological, expansible open aortic ring with a heterogenous design; the A-ring. In this study we evaluate its performance in an acute porcine model.

Methods
An 80 kg porcine model was used to evaluate aortic root motion, haemodynamics and valve performance before and after implementing the A-ring, by using epicardial echocardiography, sonomicrometry and pressure catheters. After median sternotomy, establishment of extracorporeal circulation and cardioplegic arrest, the A-ring was inserted around the aortic annulus with 6 U- sutures. The aim was to obtain a mild downsizing of the aortic annulus diameter in systole.

Results
Preliminary results show that the aortic root dynamics of the A-ring were similar to those of the native aortic root. It was able to maintain aortic root distensibility and haemodynamic performance during the cardiac cycle. Moreover, the A-ring downsized the aortic annulus diameter as intended and increased the coaptation length of the aortic valve cusps.

Conclusion
The A-ring showed physiologic expansibility comparable to that of the native aortic annulus. The results of these initial studies are promising regarding characteristics required for a supportive aortic annuloplasty ring. They underline that the A-ring has the potential to become a future adjunct for aortic valve repair- and valve-sparing aortic root procedures. This affords us with a basis for continued functional testing aiming to develop a new surgical device and create the foundation for improving the overall treatment of AI, the benefits of which will affect a broad spectrum of patients.

P6_8: Understanding the neuroprotective role of the novel HCO3-sensor RPTPγ in disease severity of ischemic stroke

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Background: Stroke is a leading cause of death and disability. Inadequate cerebral blood flow relative to metabolic demand lowers tissue oxygenation and causes acute ischemic damage followed by delayed secondary injury due to vasospasm and inflammation. Under physiological conditions, local acid-base-triggered mechanisms match cerebral blood flow to metabolic demand. These mechanisms are compromised during a stroke. The novel extracellular $HCO3^-$ sensor- RPTPγ is expressed by neurons, microvascular endothelial cells, and immune cells. The molecular mechanisms whereby RPTPγ regulates cardiovascular function remain largely unknown, however, there are some identified targets from other cell types that are known mediators of cerebral ischemic damage. Loss of function in RPTPγ variants is associated with a 7-fold increased stroke risk in humans.

Aim: This study aims to investigate the role of the $HCO3^-$sensor RPTPγ in translating acid-base disturbances in the extracellular environment of stroke lesions into altered intracellular signalling events that may confer neuroprotection.

Methods: I will induce brain ischemia in mouse models with disrupted expression of RPTPγ and explore the consequences for cerebral blood flow, neuronal metabolism, and ultimately stroke severity.

Perspective: The therapeutic interventions available during an ischemic stroke are limited. Exploring RPTPγ in ischemic stroke will provide new insights into the pathophysiological and molecular processes involved. Further, it will open new possibilities for therapies and treatments that could improve patient care.

P6_9: Lack of renoprotective effects by long-term PCSK9 and SGLT2 inhibition using alirocumab and empagliflozin in obese ZSF1 rats

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Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular disease (CVD). Despite the entry of sodium glucose cotransporter 2 (SGLT2) inhibitors, CKD persists as a medical challenge. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition reduces low-density lipoprotein (LDL)-cholesterol, a major risk factor of CVD. Interestingly, studies indicate that PCSK9 inhibition decreases proteinuria in kidney disease, complementing the reduced CVD risk.

This study aimed to validate obese ZSF1 rats as a model for the renoprotective effects of PCSK9 and SGLT2 inhibition using alirocumab and empagliflozin for 15 weeks. Obese rats revealed a significant reduction in measured glomerular filtration rate (mGFR) and increased urine albumin/creatinine ratio (UACR) during follow-up compared to lean controls. Alirocumab treatment resulted in a decline in mGFR and increased UACR compared to vehicle-treated obese rats. Immunohistochemistry showed increased fibrosis and inflammation in kidney tissue from obese rats treated with empagliflozin or alirocumab, whereas hepatic cholesterol and triglyceride levels were lowered compared to vehicle-treated obese rats. Notably, while alirocumab lowered plasma cholesterol in the beginning of the study, plasma cholesterol levels increased after 12 weeks of treatment and only a trend towards increased hepatic LDL- receptor levels was observed.

In conclusion, the PCSK9-cholesterol axis exhibited unexpected effects in obese ZSF1 rats, deviating from established biological effects of PCSK9 inhibition. These data question the predictive validity of obese ZSF1 rats as a model to study the potential of PCSK9 inhibition in kidney disease. Moreover, the renal outcomes observed with empagliflozin contradicted the renoprotective properties associated with CKD patients.

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