NORSKE ABSTRAKTER

Chronic isoproterenol treatment enhances extracellular fibrosis and postischemic diastolic dysfunction in male but not female rat heart

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Purpose: To examine the response to chronic β-adrenergic stimuli (isoproterenol, Iso) and a proposed milder response in female hearts with respect to gene expression, fibrosis and ischemic injury.

Methods: Female and male Wistar rats received either 3 mg/kg/day Iso for 7 days or sham treatment. Hearts were isolated, subjected to 30 min ischemia and 30 min reperfusion in combination with functional monitoring and thereafter harvested for gene expression and histology.

Results: Treatment effects: Heart weight and cardiomyocyte transverse cross section area increased significantly in Iso treated hearts within the same gender. Expression of the following genes was significantly changed after Iso-treatment regardless of gender. ANF, α-MHC, β-MHC, Agtr-1α, Col I-α1, Col III-α1, Fn-1. Timp-1, PKC-α, PKC-δ, PKC-α, Bcl-2, Casp3. α-MHC, PKC-α and Bcl-2 were decreased, the other increased with Iso treatment. Gender difference: There was a significant gender difference within the Iso groups in expression of fibrosis related genes Col I, Col III, Fn-1 and Timp1. These genes were overexpressed in male compared to female hearts. There was a significant gender difference in the expression of α-MHC and PKC-δ in sham hearts. No significant gender difference could be observed in the expression of the following genes: p53, BNP, Ankrd-1, TNF and e-NOS. PKC-α was increased in males compared to females regardless of treatment. End diastolic pressure after the ischemic insult increased more in Iso treated male hearts compared to female hearts. This was accompanied with increased collagen content and enhanced expression of fibrosis related genes. No significant differences could be observed in left ventricular developed pressure or in expression of different selected genes related to heart function and apoptosis.

Conclusion: Sub-chronic β-adrenergic stimulation led to enhanced expression of fibrosis related genes and higher collagen content in heart tissue in male compared to female hearts. This was accompanied by postischemic diastolic dysfunction.

The NLRP3-dependent inflammasome in relation to cardiac diseases

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Background: Myocardial infarction (MI) leads to tissue damage with subsequent sterile inflammation. Even though this inflammatory process is a prerequisite for proper tissue healing, it may also cause excessive damage if not properly regulated. Tissue damage is recognized by the innate immune system through pattern recognition receptors (PRRs) which include the transmembrane Toll-like receptors (TLRs) and the cytosolic NOD-like receptors (NLRs). The NLR NOD-Like Receptor with a Pyd domain 3 (NLRP3) is particularly important in initiating an acute sterile inflammation. NLRP3 senses necrosis or cellular damage through indirect recognition of several chemically different mediators, including ATP and mono sodium urate crystals, as well as oxidative stress. Upon activation, NLRP3 forms multiprotein complexes with ASC and caspase-1 which proteolytically activate pro-IL-1β. The role of NLRP3 in the heart is not known. Our objective was to investigate the expression and function of NLRP3 in cardiac fibroblasts, as well as the role of NLRP3s in ex-vivo ischemia-reperfusion injury.

Methods and results: In a mouse MI model, NLRP3 and IL-1β mRNA was increased in the non-ischemic and in particular the ischemic parts of the left ventricle. The TLR4 ligand lipopolysaccharide (LPS) and other TLR agonists markedly increased the gene expression of both NLRP3 and IL-1β in adult cardiac fibroblasts. However, a prerequisite for IL-1β release was the addition of ATP, an activator of the NLRP3 inflammasome. Confocal microscopy confirmed the formation of "inflammasome-like" structures in cardiac fibroblasts upon ATP stimulation. Importantly, IL-1β release was not found in fibroblasts from NLRP3 deficient mice or when the activity of caspase-1 was inhibited, demonstrating a pivotal role for the NLRP3 inflammasome in the post-translational activation of IL-1β. Finally, we examined Langendorff-perfused hearts from NLRP3−/−- and wild-type mice. Global ischemia and

reperfusion in NLRP3 deficient hearts resulted in markedly lower left ventricular end-diastolic pressure and a reduced infarct size as compared to Wt hearts.

Conclusions: We demonstrate, for the first time, a functional NLRP3 inflammasome in cardiac fibroblasts. The NLRP3 inflammasome represents an important contributor to inflammation and tissue damage during myocardial ischemia-reperfusion injury.

Krill oil attenuates cardiac remodelling after myocardial infarction in rats

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Purpose: Supplement with n-3 polyunsaturated fatty acids (PUFA) has previously been shown to improve cardiac function and to decrease mortality after myocardial infarction (MI) and in heart failure. Data indicate that the composition of n-3 PUFA may influence myocardial structure and function. The biochemical construction of n-3 PUFA varies between different marine sources. Krill oil, unlike fish oil supplements, contains the major part of the n-3 PUFA in the form of phospholipids. This study investigated the effects of krill oil on cardiac remodelling and function in rats after MI.

Methods: Rats were randomised to pre-treatment with krill oil or placebo for 14 days before induction of MI. Sham-operated rats served as controls. Seven days post-MI the rats were examined with echocardiography, and MI rats in the placebo group were further randomised to treatment with krill oil or placebo. The subsequent three groups of rats were treated for 7 weeks before examination by echocardiography. Further, composition of FA and expression of selected genes known to be involved in cardiac remodelling were examined in the non-infarcted left ventricular (LV) tissue.

Results: Significant cardiac remodelling and reduced cardiac function were seen in the MI-placebo rats. Rats treated with krill oil had a significantly higher proportion of n-3 PUFA and an increased n-3/n-6 PUFA ratio in the LV tissue, though no difference in such alteration was seen between the two krill oil groups. However, only MI rats pre-treated with krill oil displayed significant attenuation of LV dilatation and attenuated increases in LV and lung weight. There was no effect on LV function. These beneficial effects on cardiac remodelling were mirrored by significant decreased LV levels of mRNAs encoding classical markers of LV stress (ANP), matrix remodelling (TIMP-1 and TGF-β), and inflammation (IL-β and IL-6).

Conclusions: Supplement with krill oil led to a proportional increase of n-3 PUFA in myocardial tissue and pre-treatment with krill oil attenuated LV remodelling after MI.

Changes in symptom and quality-of-life assessments correlate strongly and consistently with changes in functional capacity in patients with heart failure

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Purpose: Clinical status in heart failure is conventionally assessed by the patient`s own perception of their symptoms and quality of life (QoL) and a measure of functional capacity. The former may be measured with tools such as EQ-5D, the Kansas City Cardiomyopathy Questionnaire (KCCQ), Patient Global Assessment (PGA) and the latter by 6 minute walk test (6MWT) performance. The FAIR-HF trial demonstrated that treatment with intravenous ferric carboxymaltose (FCM) in patients with heart failure significantly improved symptoms, QoL and functional capacity. This analysis assessed the correlations between the changes in the measures of QoL and the changes in the measure of functional capacity.

Methods: The FAIR-HF trial randomized 459 patients with chronic heart failure (impaired left ventricular ejection fraction) and iron deficiency, with or without anaemia, to FCM or placebo (2:1). Patients were assessed using the KCCQ,

the EQ-5D and the 6MWT at baseline and after 4, 12, 24 weeks. PGA, which reports the patient`s own evaluation of any change in overall status, was recorded at 4, 12 and 24 weeks.

Results: The data demonstrate highly significant correlations between symptoms and functional capacity at all time points ($p < 0.0001$). Importantly, changes in PGA, KCCQ and EQ-5D correlate strongly and consistently with changes in 6MWT performance. The strength of these correlations increased over time and were strongest at 24 weeks.

Conclusion: The data provide consistent evidence that the symptom and QoL assessments correlate strongly with changes in functional capacity as measured by the 6MWT and demonstrate internal validation of these clinically meaningful endpoints.

Correlations with 6MWT changes			
	4 weeks	12 weeks	24 weeks
ΔKCCQ	0.316*	0.415*	0.474*
n=	414	409	390
ΔEQ-5D	0.308*	0.424*	0.472*
n=	413	408	390
PGA	−0.433*	−0.501*	−0.561*
n=	424	421	399
*p < 0.0001			

Sex- and age -based differences in cardiac tolerance to ischemia-reperfusion injury

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Purpose: Gender and age play a important roles in normal cardiac physiology as well as in cardiac pathophysiology. The aim of this study was to improve the understanding of gender and age in cardiac tolerance to ischemia.

Methods: Male or female C57Bl6 mice were evaluated at the age of 3 months (young) or 9 months (old)(n = 10 in each group). Hearts were isolated and subjected to Langendorff-perfusion with induced global ischemia (30 min) followed by reperfusion (60 min). Left ventricular systolic (LVSP), and end diastolic pressures (LVEDP), heart rate (HR) and coronary flow (CF) were measured, and left ventricular developed pressure (LVDP=LVSP-LVEDP) and contractility (+dp/dt) calculated. Myocardial infarct size was determined by triphenyltetrazolium chloride staining.

Results: The basal cardiac performance was similar between groups. During reperfusion, LVEDP tended to be higher in both male groups than female groups, but not significantly so. LVDP in young females was improved compared with young males ($p < 0.05$), but this beneficial effect disappeared in old females. Young female mouse hearts had significantly improved +dp/dt compared with both male groups as well as old females ($p < 0.05$). CF tended to be higher in males than females. Infarct size was not significantly different between groups.

Conclusions: These findings confirm that age and gender play a role for cardiac tolerance to ischemia, where the impact on heart function was more evident than an infarct-sparing effect. The reasons for this will be subjected to further investigations.

Left atrial strain by speckle tracking echocardiography is a new noninvasive predictor of cardiac death or need of heart transplantation in patients with moderate to severe heart failure

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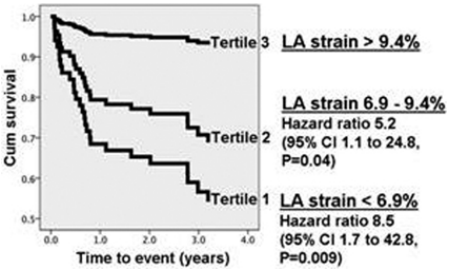
Background: We have recently demonstrated a close inverse relationship between left atrial (LA) stretching during its filling, measured as peak LA strain by speckle tracking echocardiography (STE), and pulmonary capillary wedge pressure, an indirect measure of left ventricular end-diastolic pressure. In this study we investigated if LA strain was an independent predictor of cardiac events (death or heart transplantation) in patients with moderate to severe heart failure.

Methods: We prospectively included 99 patients with ischemic or dilated cardiomyopathy (NYHA II-IV). Peak LA strain by STE, LA area by planimetry, LV ejection fraction (EF) by the Simpson's method, and E/e' by tissue-Doppler imaging were measured. Time to event (Cox model) was adjusted for age, NT-proBNP, LA area, LVEF and E/e' in addition to LA strain.

Results: Median follow-up time was 3.0 years. There were 28 events and mean time to event was 1.2±1.1 years. The Cox analysis demonstrated that only LVEF (P = 0.01), NT-proBNP (P = 0.006) and LA strain (P = 0.005) were independent predictors of events: For 1 unit increase in LA strain there was a 16% reduction in the adjusted relative risk for events (Hazard ratio, 0.84, 95% CI 0.74-0.95). Importantly, for the lowest tertile of LA strain there was an almost 9-fold increase in the adjusted relative risk for

events, when compared to the highest tertile (Figure 1).



Conclusion: In patients with moderate to severe heart failure, LA strain by STE is a strong independent predictor of death or need of heart transplantation. This finding suggests that LA strain by STE might be a useful marker in the risk stratification of patients with heart failure.

N-terminal pro-B-type natriuretic peptide is a marker of reversible myocardial dysfunction after non-ST-elevation acute coronary syndrome

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Purpose: In acute coronary syndrome (ACS), myocardial function is often impaired. Some of this impairment may be due to reversible phenomena, including myocardial stunning. N-terminal pro B-type natriuretic peptide (NT-proBNP) and troponin T (TnT) are released in proportion to the size of the myocardial injury. Our aim was to investigate the association between plasma levels of these biomarkers and the development in left ventricular (LV) function and size after non-ST-elevation (NSTE-) ACS.

Methods: In 119 patients, age 58±9 years, admitted for NSTE-ACS, echocardiography and blood sampling were performed at baseline and at follow-up after 8±3 months. LV systolic function was assessed by speckle tracking echocardiography as global longitudinal strain (GLS), negative values representing myocardial shortening. In 50 patients, final infarct size was determined by magnetic resonance imaging. Baseline levels of NT-proBNP and TnT were determined by high-sensitive assays, and their association with myocardial functional recovery, LV intra ventricular volumes, and infarct size were determined by linear regression.

Results: Levels of TnT and NT-proBNP were associated with baseline myocardial function, described as GLS ($r = 0.46$; $p < 0.001$ and $r = 0.42$; $p < 0.001$, respectively). Neither biomarker was associated with LV volumes. NT-proBNP was associated with the decline in GLS between baseline and follow-up: The higher the NT-proBNP, the larger the myocardial recovery ($r = -0.40$; $p < 0.001$: illustration 1). This association was independent of the functional impairment at baseline, infarct size and time to reperfusion.

Conclusion: In NSTE-ACS, elevated levels of NT-proBNP are independently associated with improved myocardial performance after 8 months.